Department of Medicine, Division of Cardiology
Helsinki University Central Hospital
Helsinki, Finland

DETERMINANTS OF CORONARY AND CAROTID ATHEROSCLEROSIS IN FINNISH PATIENTS WITH CLINICALLY SUSPECTED CORONARY ARTERY DISEASE

A Quantitative Angiography and Ultrasound Study

Marit Granér

ACADEMIC DISSERTATION

To be presented,
with the permission of the Medical Faculty of the University of Helsinki,
for public examination in Auditorium 2, Meilahti Hospital,
on May 18th 2007, at 12 noon.

HELSINKI 2007

Supervisors

Professor Marja-Riitta Taskinen, MD
Department of Medicine, Division of Cardiology
Helsinki University Central Hospital
Helsinki, Finland

and

Docent Mikko Syvänne, MD
Department of Medicine, Division of Cardiology
Helsinki University Central Hospital
Helsinki, Finland

Reviewers

Professor Timo Strandberg, MD
Department of Public Health Science and General Practice
University of Oulu
Oulu, Finland

and

Docent Raimo Kettunen, MD
Department of Medicine
Päijät-Häme Central Hospital
Lahti, Finland

Opponent

Professor Juhani Airaksinen, MD
Department of Medicine
University of Turku
Turku, Finland

ISBN 978-952-92-1845-5 (paperback)
ISBN 978-952-10-3822-8 (PDF)

University Printing House
Helsinki 2007

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Granér M, Varpula M, Kahri J, Salonen RM, Nyyssönen K, Nieminen MS, Taskinen MR, Syvänne M. Association of carotid intima-media thickness with angiographic severity and extent of coronary artery disease. *Am J Cardiol* 2006;97:624-629.

II Granér M, James RW, Kahri J, Nieminen MS, Syvänne M, Taskinen MR. Association of paraoxonase-1 activity and concentration with angiographic severity and extent of coronary artery disease. *J Am Coll Cardiol* 2006;47:2429-2435.

III Granér M, Kahri J, Nakano T, Sarna SJ, Nieminen MS, Syvänne M, Taskinen MR. Impact of postprandial lipemia on low-density lipoprotein (LDL) size and oxidized LDL in patients with coronary artery disease. *Eur J Clin Invest* 2006;36:764-770.

IV Granér M, Syvänne M, Kahri J, Nieminen MS, Taskinen MR. Insulin resistance as predictor of the angiographic severity and extent of coronary artery disease. *Ann Med* 2006;1-8:PrEview.

V Granér M, Kahri J, Varpula M, Salonen RM, Nyyssönen K, Jauhiainen M, Nieminen MS, Syvänne M, Taskinen MR. Apolipoprotein E polymorphism is associated with both carotid and coronary atherosclerosis in patients with coronary artery disease. *In press.*

ABBREVIATIONS

Apo	apolipoprotein
ABCA1	ATP-binding cassette transporter A1
CAD	coronary artery disease
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CM	chylomicron
CVD	cardiovascular disease
DM	diabetes mellitus
EBCT	electron beam computed tomography
ELISA	enzyme-linked immunosorbent assay
FW	far wall
HDL	high-density lipoprotein
HOMA	the homeostasis model assessment
IDL	intermediate-density lipoprotein
IMT	intima-media thickness
IR	insulin resistance
IVUS	intra-vascular ultrasound
LCAT	lecithin:cholesterol acyltransferase
LDL	low-density lipoprotein
LOX-1	lecitin-like oxidized low-density lipoprotein receptor-1
Lp	lipoprotein
MRA	magnetic resonance angiography
MSCT	multislice computed tomography
NW	near wall
OxLDL	oxidized low-density lipoprotein
PDS	percent diameter stenosis
PLTP	phospholipid transfer protein
PON1	paraoxonase-1
QCA	quantitative coronary angiography
RLP	remnant lipoprotein particle
RLP-C	the cholesterol content of remnant lipoprotein particle
Sf	Svedberg flotation unit
TG	triglyceride
TRL	triglyceride-rich lipoprotein
VLDL	very low-density lipoprotein

ABSTRACT

Background. Cardiovascular disease (CVD) remains the most serious threat to life and health in industrialized countries. Atherosclerosis is the main underlying pathology associated with CVD, in particular coronary artery disease (CAD), ischaemic stroke, and peripheral arterial disease. Risk factors play an important role in initiating and accelerating the complex process of atherosclerosis. Most studies of risk factors have focused on the presence or absence of clinically defined CVD. Less is known about the determinants of the severity and extent of atherosclerosis in symptomatic patients.

Aims. To clarify the association between coronary and carotid artery atherosclerosis, and to study the determinants associated with these abnormalities with special regard to novel cardiovascular risk factors.

Subjects and methods. Quantitative coronary angiography (QCA) and B-mode ultrasound were used to assess coronary and carotid artery atherosclerosis in 108 patients with clinically suspected CAD referred for elective coronary angiography. To evaluate anatomic severity and extent of CAD, several QCA parameters were incorporated into indexes. These measurements reflected CAD severity, extent, and overall atheroma burden and were calculated for the entire coronary tree and separately for different coronary segments (i.e., left main, proximal, mid, and distal segments). Maximum and mean intima-media thickness (IMT) values of carotid arteries were measured and expressed as mean aggregate values. Furthermore, the study design included extensive fasting blood samples, oral glucose tolerance test, and an oral fat-load test to be performed in each participant.

Results. Maximum and mean IMT values were significantly correlated with CAD severity, extent, and atheroma burden. There was heterogeneity in associations between IMT and CAD indexes according to anatomical location of CAD. Maximum and mean IMT values, respectively, were correlated with QCA indexes for mid and distal segments but not with the proximal segments of coronary vessels. The values of paraoxonase-1 (PON1) activity and concentration, respectively, were lower in subjects with significant CAD and there was a significant relationship between PON1 activity and concentration and coronary atherosclerosis assessed by QCA. PON1 activity was a significant determinant of severity of CAD independently of HDL cholesterol. Neither PON1 activity nor concentration was associated with carotid IMT. The concentration of triglycerides (TGs), triglyceride-rich lipoproteins (TRLs), oxidized LDL (oxLDL), and the cholesterol content of remnant lipoprotein particle (RLP-C) were significantly increased at 6 hours after intake of an oral fatty meal as compared with fasting values. The mean peak size of LDL remained unchanged 6 hours after the test meal. The correlations between total TGs, TRLs, and RLP-C in fasting and postprandial state were highly significant. RLP-C correlated with oxLDL

both in fasting and in fed state and inversely with LDL size. In multivariate analysis oxLDL was a determinant of severity and extent of CAD. Neither total TGs, TRLs, oxLDL, nor LDL size were linked to carotid atherosclerosis. Insulin resistance (IR) was associated with an increased severity and extent of coronary atherosclerosis and seemed to be a stronger predictor of coronary atherosclerosis in the distal parts of the coronary tree than in the proximal and mid parts. In the multivariate analysis IR was a significant predictor of the severity of CAD. IR did not correlate with carotid IMT. Maximum and mean carotid IMT were higher in patients with the apoE4 phenotype compared with subjects with the apoE3 phenotype. Likewise, patients with the apoE4 phenotype had a more severe and extensive CAD than individuals with the apoE3 phenotype.

Conclusions. 1) There is an association between carotid IMT and the severity and extent of CAD. Carotid IMT seems to be a weaker predictor of coronary atherosclerosis in the proximal parts of the coronary tree than in the mid and distal parts. 2) PON1 activity has an important role in the pathogenesis of coronary atherosclerosis. More importantly, the study illustrates how the protective role of HDL could be modulated by its components such that equivalent serum concentrations of HDL cholesterol may not equate with an equivalent, potential protective capacity. 3) RLP-C in the fasting state is a good marker of postprandial TRLs. Circulating oxLDL increases in CAD patients postprandially. The highly significant positive correlation between postprandial TRLs and postprandial oxLDL suggests that the postprandial state creates oxidative stress. Our findings emphasize the fundamental role of LDL oxidation in the development of atherosclerosis even after inclusion of conventional CAD risk factors. 4) Disturbances in glucose metabolism are crucial in the pathogenesis of coronary atherosclerosis. In fact, subjects with IR are comparable with diabetic subjects in terms of severity and extent of CAD. 5) ApoE polymorphism is involved in the susceptibility to both carotid and coronary atherosclerosis.

1. INTRODUCTION

Atherosclerosis is a generalized disease of the arterial wall, which may progress or regress depending on a plethora of factors. The classic risk factors for atherosclerosis in the general population are age, male gender, family history of premature cardiovascular disease (CVD), diabetes mellitus (DM), hypertension, smoking, high total and low-density lipoprotein (LDL) cholesterols, low high-density lipoprotein (HDL) cholesterol, and obesity (Fruchart et al. 2004). More recently, several novel risk factors, such as small, dense LDL, oxidized LDL (oxLDL), insulin resistance (IR), lipoprotein(a) [Lp(a)], apolipoprotein (apo)E4 isoform, HDL-bound paraoxonase-1 (PON1), hypertriglyceridemia, and triglyceride-rich lipoproteins (TRLs) have been linked with an increased risk of CVD (Mackness et al. 2001, Carmena et al. 2004, Fruchart et al. 2004, Song et al. 2004).

Although risk factors play an important role, emerging evidence suggests that the impact of these on initiating and accelerating the complex process of atherosclerosis in different arterial territories is not similar (Faxon et al. 2004). Furthermore, most studies of cardiovascular risk factors have focused on the presence or absence of clinically defined coronary artery disease (CAD). Less is known about the determinants of the severity and extent of coronary atherosclerosis in symptomatic patients. The severity and extent of CAD is important for prognosis, since people with no or minimal narrowings have an excellent prognosis, whereas those with left main coronary disease or triple-vessel disease have an ominous clinical course without intervention.

Atherosclerosis often remains clinically "silent" for many years, and the first clinical manifestation of atherosclerotic disease is frequently a major cardiovascular event, such as myocardial infarction, stroke, or sudden death. Over recent decades the interest in cardiovascular epidemiology has broadened from studies on causes and consequences of elevated cardiovascular risk factors to include research on causes and consequences of atherosclerosis and associated arterial wall abnormalities. One of the underlying reasons was that established cardiovascular risk factors were insufficiently accurate in identifying those individuals who will suffer from CVD in future.

Because angiographic assessment of the coronary arteries is expensive and not without risk, ultrasonographic measurement of intima-media thickness (IMT) of easily accessible carotid arteries has been advocated as surrogate marker for coronary atherosclerosis. An increase in IMT of the carotid artery has been associated with established risk factors of CVD (Heiss et al. 1991, Salonen et al. 1991, Davis et al. 2001), presence of CAD (Craven et al. 1990, Crouse et al. 1995), as well as the occurrence of cardiovascular events (Lorenz et al. 2007).

Against this background the present study was undertaken to clarify, in a cohort of patients with clinically suspected CAD, the association between ultrasonically determined carotid atherosclerosis and anatomic distribution of coronary atheroma based on a computer-assisted analysis of coronary angiograms, and to study the determinants associated with these abnormalities with special regard to novel cardiovascular risk factors.

2. REVIEW OF THE LITERATURE

2.1. Pathogenesis of atherosclerotic vascular disease

Atherosclerosis is a pathological condition that underlies several important adverse vascular conditions including CAD, cerebrovascular disease, and peripheral arterial disease. The lesions of atherosclerosis occur principally in large and medium-sized arteries. The arterial wall consists of three anatomically distinct layers: the intima, media, and adventitia. The intima is separated from the media by the internal elastic lamina, which is considered to be part of the media. The external elastic lamina separates the media from the adventitia (Stehbens 1995). Atherosclerosis is predominantly a disease of the intima and occurs at so-called lesion-prone sites near the vessel branches and bifurcations at which local factors such as variable blood flow and low and oscillating shear influence the atherogenic process (Gotto et al. 1999).

Traditionally, atherosclerotic changes in the arterial wall are divided into six characteristic developmental stages (Figure 1). The earliest sign of atherosclerosis, the type I lesions, comprises formation of small isolated groups of macrophages filled with lipid droplets (foam cells). Type II lesions, grossly designated as fatty streaks, are characterized by closely packed lipid-laden macrophages, smooth muscle cells, and lymphocytes. By the time of puberty, a variable number of type I and type II lesions are present in nearly everyone to a variable extent. They may regress temporarily or permanently. The progression beyond the type II morphology requires an additional stimulus, i.e. risk factor for the development of atherosclerosis. Type III lesions, also known as preatheromas, form the bridge between early and advanced lesions. In addition to the features of type II lesions, type III lesions contain a mixture of cell remnants and lipid droplets packed between smooth muscle cells of the deep (musculoelastic) layer of the intima, forming small separate pools in the spaces between the cells. Accumulation of this extracellular lipid constitutes the onset of progression beyond the earlier minimal changes. Lesion types I through III do not thicken the arterial wall appreciably and therefore do not narrow the lumen or obstruct blood flow (Stary et al. 1994).

By the end of the third decade of life, the first potentially clinical lesions may be found at highly susceptible arterial locations; these are known as type IV lesions or atheroma. The cap of the lesion, i.e. the tissue layer between the lipid core and the endothelial surface, varies in thickness and contains macrophages, smooth muscle cells, and inflammatory cells (Ross 1999). When the cap covering a lipid core undergoes a substantial increase in fibrous tissue, the lesions are labeled type V. The narrowing (loss) of the vascular lumen is a prominent feature of type V lesions. When lesions (generally types IV and V) include surface disruption, hematoma, thrombus or all of these together, they are called type VI or complicated lesions (Stary et al. 1995).

An atherosclerotic plaque, which is prone to rupture, is characterized by a large, lipid-rich atheromatous core and a thin fibrous cap. Moreover, the amount of inflammatory cells in the plaque is increased (Ross 1999, Hansson 2005, Falk 2006). In the event of erosion or rupture of atherosclerotic plaque, exposure of its thrombogenic content into the lumen of the artery activates platelets and the coagulation system leading to formation of a thrombus, which may partially or even totally occlude the lumen of the coronary artery (Falk 2006).

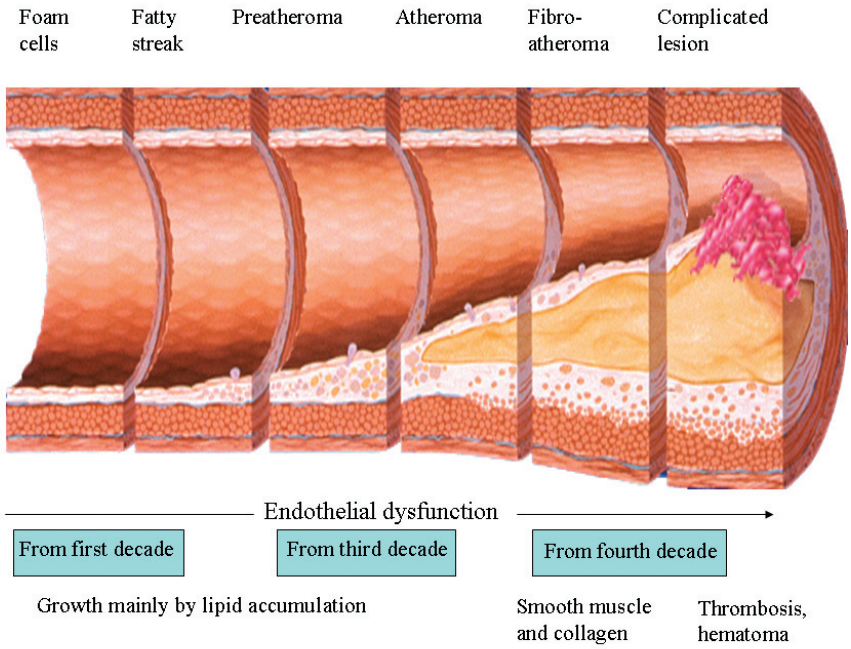


Figure 1. Evolution and progression of human atherosclerotic lesions. Modified from Stary et al. (1995).

CAD is largely not a disease of the lumen but an abnormality of the vessel wall. Regardless of the size of the lumen, if a plaque ruptures and a thrombus develops, a patient can experience an acute coronary syndrome or even sudden cardiac death. Thus, the traditional model (Figure 1) of the disease, in which the plaque develops in the vessel wall over many years, gradually narrowing the lumen to produce symptoms, is not completely correct. A more accurate model of atherosclerosis was originally described in a necropsy study by Glagov et al. (1987), who showed that, in the early course of atherosclerosis, coronary "remodeling" of the vessel wall enables patients to develop large atherosclerotic plaques without reduction in lumen size. According to Glagov et al. (1987), lumen reduction does not occur until the plaque occupies >40% of the total vessel cross-sectional area. More recently, this phenomenon of "arterial remodeling" has been confirmed in vivo by the use of intracoronary

ultrasound imaging (De Franco et al. 2001). Positive remodeling is defined as a compensatory outward expansion of local vessel size in response to plaque accumulation. Negative remodeling is described as the shrinkage of local vessel size in association with the development of CAD (De Franco et al. 2001). Intracoronary ultrasound studies have demonstrated an association between the direction of remodeling and clinical presentation. Acute coronary syndromes are more commonly characterized by positive than negative remodeling, whereas lesions accompanied by negative remodeling are associated with stable angina pectoris (Schoenhagen et al. 2001). Further, remodeling has been shown to occur much less in distal coronary artery segments than in proximal segments (Burke et al. 2002).

The pathophysiology of vascular remodeling is not fully understood. However, available data suggest that remodeling is initiated by changes in hemodynamic conditions (flow, wall stretch, shear stress) and humoral factors (cytokines, vasoactive substances) (Schoenhagen et al. 2001). Histopathologic studies have revealed that plaque components, such as macrophage burden, lipid core size, calcium (in fibrous plaque and lipid core), and medial atrophy are strongly asscoiated with positive remodeling, whereas fibrous areas are negatively correlated with remodeling.

2.2. A short overview of lipoprotein metabolism

2.2.1. The major lipoprotein species

The major fatty substances or lipids in the plasma are fatty acids, triglycerides (TGs), cholesterol (free and esterified cholesterol), and phospholipids. They are important in maintaining the structure of the cell membrane (cholesterol, phospholipids), and as substrates for steroid hormone synthesis (cholesterol) and energy metabolism (TGs, fatty acids). Since lipids are insoluble in the aqueous milieu of blood plasma, they have to be packaged into particles known as lipoproteins (Gotto et al. 1986). Lipoproteins consist of a hydrophobic core of cholesteryl esters and TGs surrounded by a hydrophilic surface of free (unesterified) cholesterol, phospholipids, and apolipoproteins.

Apolipoproteins maintain the structural integrity of lipoproteins and direct their metabolic interactions with cell-surface receptors, hydrolytic enzymes, and lipid transport proteins. As outlined in Table 1, plasma lipoproteins are traditionally classified, according to the density at which they float during ultracentrifugation, into five categories: chylomicrons (CMs), very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), LDLs, and HDLs. Some lipoprotein subclasses can be further separated by particle size, electrophoretic mobility, and apolipoprotein content.

Table 1. Physiochemical properties, lipid and apolipoprotein composition of the major human plasma lipoproteins.

	Exogenous lipids	Endogenous lipids			
	CM	VLDL	IDL	LDL	HDL
Density range (g/mL)	<0.96	0.96-1.006	1.006-1.019	1.019-1.063	1.063-1.210
Particle size (diameter, nm)	75-1200	30-80	25-35	18-25	5-12
Flotation rate (Sf)*	400-10,000	20-400	12-20	0-12	
Composition (%)					
TG	86	55	23	6	5
PL	7	18	19	22	33
CE	4	12	29	42	17
FC	2	7	9	8	5
Protein	2	8	19	22	40-55
Major apolipoprotein component(s)	A-I, A-II, A-IV, B-48, C†, E	B-100, C†, E	B-100, C†, E	B-100	A-I, A-II, A-III, C†, E
Source	Intestine	Liver	Lipolysis of VLDL	Lipolysis of VLDL, via IDL	Liver, intestine; lipolysis of CM & VLDL

CM, chylomicron; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; PL, phospholipid; CE, cholesteryl ester; FC, free cholesterol

*Svedberg flotation rate at 26°C and d 1.063 g/ml (not applicable to HDL).

†There are three apoC peptides: apoC-I, apoC-II, and apoC-III.

Adapted from Gotto et al. (1986) and Ginsberg (1990).

2.2.2. Triglyceride-rich lipoprotein

2.2.2.1. Definition and characteristics of chylomicrons

Dietary fat is digested in the small intestine and absorbed into the enterocytes. Within the enterocytes, the exogenous free fatty acids, glycerols, and monoacylglycerols are packaged as TGs together with cholesteryl esters, phospholipids, and proteins into CM particles (Green et al. 1981, Bisgaier et al. 1983).

CMs are secreted into the intestinal lymph and transported into the bloodstream via the thoracic duct. The secreted CMs interact with HDLs, donating apoA-I, apoA-II, apoA-IV, and phospholipids and receiving apoCI, apoCII, apoCIII, and apoE (Gotto et al. 1986). ApoB-48, a specific apolipoprotein constituent of CMs, remains within the CM particle throughout its life span. Lipoprotein lipase removes TGs from the CM particles to be utilized in peripheral tissues. Thereafter, CMs be-

come remnant particles, which are taken up by the hepatic cells. The cellular uptake of CM remnants is mediated by multiple interactions of apoE and lipoprotein lipase with heparan sulfate proteoglycans, the LDL receptor, and the LDL receptor-related protein (Beisiegel et al. 1991, Heeren et al. 2001) (Figure 2).

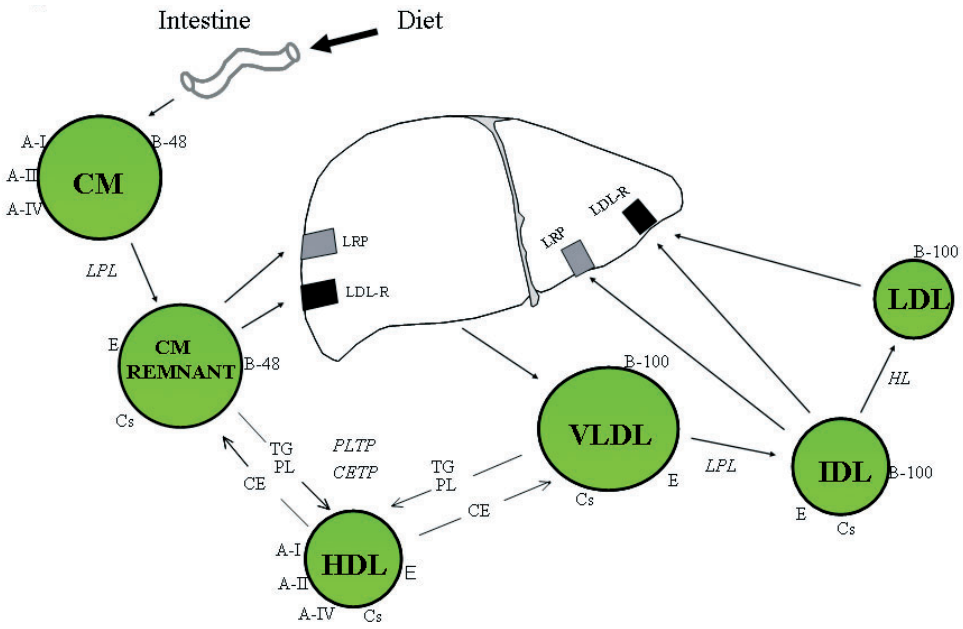


Figure 2. Schematic illustration of lipoprotein metabolism. For explanation, see text (chapter 2.2.2.). A-I, A-II, A-IV, B-48, B-100, E, apolipoproteins; Cs, apolipoproteins C-I, C-II, C-III; CM, chylomicron; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; LDL-R, LDL receptor; LRP, LDL receptor-related protein; LPL, lipoprotein lipase; HL, hepatic lipase; TG, triglyceride; PL, phospholipid; CE, cholesteryl ester; PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein.
Modified and used with permission from Kati Ylitalo, Doctoral Thesis 2001.

2.2.2.2. Defintion and characteristics of very low-density lipoproteins

VLDLs are secreted from the liver and contain cholesterol, TGs, apoB-100, apoE, and apoCs. The apoE and some of the apoCs are transferred from HDL to VLDL. ApoB-100, synthesized in the liver, is the main apolipoprotein of VLDL. Likewise the catabolic pathways of CMs, VLDLs are hydrolyzed by lipoprotein lipase to VLDL remnants. As a result of TG depletion, VLDL remnants can be removed directly by the liver or converted into IDL particles. IDLs are further hydrolyzed by hepatic lipase and lipoprotein lipase into LDL (Demant et al. 1988) (Figure 2).

By definition, the term remnant refers to an apoB-containing lipoprotein that has delivered some, or major parts, of its original TG content to peripheral tissues by means of lipoprotein lipase-mediated lipolysis (Karpe 1999). In a sense, all apoB-containing lipoproteins are remnants because the intestinally derived CMs and liver-derived VLDLs are nascent particles that are rapidly catabolized. It has been shown that the half-life of the removal of intravenously injected CMs from circulation varies from 5 to 13 minutes (Nestel 1964).

Taken together, TRLs are highly heterogeneous in size, density, and composition. They comprise intestinally derived apoB-48 containing CMs, liver-derived apoB-100 containing VLDLs, and their remnants (Havel 1994). The capacity of TRLs to enter the arterial intima is inversely related to the size of the lipid particle. Whereas CMs and large VLDLs (Svedberg flotation unit [Sf] 60-100) are unable to pass through the endothelial layer, smaller VLDLs (Sf 20-60) and IDLs (Sf 12-20) can enter the subintimal space (Nordestgaard 1996).

2.2.3. Low-density lipoprotein

2.2.3.1. Structure of the LDL particle

LDL is a spherical particle composed of a central core of cholesteryl esters and TGs. It is surrounded by a polar coat comprising unesterified cholesterol, phospholipids, and an essential structural protein called apoB-100. Each LDL particle has one apoB molecule. ApoB-100 binds to the LDL receptor and is therefore a crucial link in the normal pathway by which LDL is removed from plasma.

About half of the fatty acids in LDL are polyunsaturated fatty acids, mainly linoleic acid with minor amounts of arachidonic and docosahexaenoic acid. These polyunsaturated fatty acids are protected against free radical attack and oxidation by antioxidants, primarly α-tocopherol (~ six molecules per LDL particle), with minor amounts of γ-tocopherol, carotenoids, cryptoxanthin, and ubiquinol-10. The amount of polyunsaturated fatty acids and antioxidants varies significantly within individuals, resulting in a great variation in LDL oxidation susceptibility (Mertens et al. 2001).

2.2.3.2. LDL metabolism

Most LDL particles originate from the metabolism of TRLs. In this VLDL-IDL-LDL cascade, the particle is depleted of TGs and loses most of the associated apolipoproteins, except for apoB-100. A small amount of LDL can also be synthesized directly by the liver. LDLs are removed from the plasma mainly via receptor-mediated endocytosis by LDL receptors on the surface of liver cells. The number of LDL receptors expressed by liver cells is controlled by negative-feedback regulation. When the concentration of cholesterol in hepatocytes rises, transcription of the LDL receptor gene is suppressed, and LDL is retained in plasma. In contrast,

when hepatic cholesterol level falls, LDL receptor gene transcription is induced, LDL is taken up more rapidly, and the amount of LDL in plasma falls (Brown et al. 1986).

2.2.3.3. LDL subfractions

LDLs are a heterogenous class of particles that can be fractionated into subclasses by analytical ultracentrifugation (Lindgren et al. 1969), density gradient ultracentrifugation (Krauss et al. 1992), gradient gel electrophoresis (Krauss et al. 1992), nuclear magnetic resonance (Otvos et al. 1992), or size exclusion chromatography (Marais 2000). Analytical ultracentrifugation, the original gold standard, measures the flotation velocity of LDL in a gravitational field; the faster the velocity, the more lipid rich the LDL. This method is currently available only in a few research laboratories worldwide. The simpler and less labor intensive density gradient ultracentrifugation separates LDL into three fractions: large, buoyant LDL (1.025<d<1.034 g/mL), intermediate size LDL (1.034<d<1.044 g/mL), and small, dense LDL (1.044<d<1.060 g/mL) (Griffin et al. 1990). The commonly used gradient gel electrophoresis yields two different LDL subclasses: pattern A and pattern B. In pattern A, the major peak of LDL particle diameter is greater than 25.5 nm. In pattern B, the major peak of LDL particle diameter is less or equal to 25.5 nm (Austin et al. 1988). Gradient gel electrophoresis has been extensively validated using ultracentrifugation. Unlike ultracentrifugation, gradient gel electrophoresis does not quantify the concentration of a particular species of LDL. Nuclear magnetic resonance is the most convenient and rapid method for measuring LDL size and concentration. However, validation studies using nuclear magnetic resonance and ultracentrifugation on the same samples in large populations have not been published (Sacks et al. 2003).

2.2.4. High-density lipoprotein

2.2.4.1. Structure of the HDL particle

HDLs are the smallest and densest of the major lipoprotein classes. They consist of a hydrophobic core surrounded by a surface of phospholipids, free cholesterol, and apolipoproteins. ApoA-I and apoA-II are the main structural proteins in HDL, accounting for approximately 70% and 20%, respectively, of total HDL protein mass. As a result, plasma apoA-I concentrations correlate closely with those of plasma HDL cholesterol. ApoA-IV, apoA-V, apoC-I, apoC-II, apoC-III, apoD, apoE, and apoJ are found in lower amounts in mature HDLs. Some HDL-associated proteins are enzymatically active, e.g. lecithin:cholesterol acyltransferase (LCAT), PON1, platelet activating factor-acetylhydrolase, and glutathione peroxidase, or provide a transport vehicle for other plasma proteins that are involved in lipid metabolism, including cholesteryl ester- and phospolipid transfer proteins (CETP, PLTP) (Barter et al. 2003) (Figure 3).

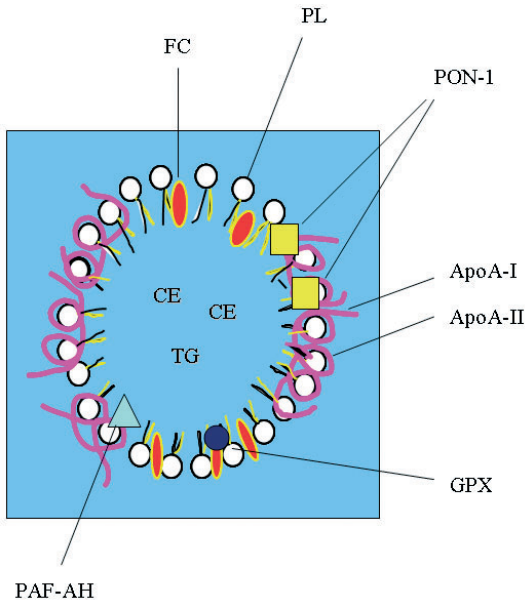


Figure 3. Structure of the HDL particle. For explanation, see text (chapter 2.2.4.). PL, phospholipid; FC, free cholesterol; CE, cholesterol ester; TG, triglyceride; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; PAF-AH, platelet activating factor-acetylhydrolase; PON-1, paraoxonase-1; GPX, glutathione peroxidase.

2.2.4.2. HDL subfractions and apolipoproteins

The HDL fraction in human plasma is heterogeneous in terms of shape, size, density, protein composition, and surface charge. When isolated on basis of density by ultracentrifugation, the major subpopulations of HDLs are HDL_2 (1.063<d<1.125 g/mL) and HDL_3 (1.125<d<1.210 g/mL) (Havel et al. 1955). According to particle sizes, HDL can be divided into HDL_{2b} (12.9-9.7 nm), HDL_{2a} (9.7-8.8 nm), HDL_{3a} (8.8-8.2 nm), HDL_{3b} (8.2-7.8 nm), and HDL_{3c} (7.8-7.2 nm) in non-denaturating polyacrylamide gradient gel electrophoresis (Nichols et al.1986). By immunoaffinity chromatography HDL can also be classified on the basis of its main apolipoproteins to particles containing only apoA-I (LpA-I) and those containing both apoA-I and apoA-II (LpA-I/A-II). Most of the LpA-I is found in HDL_2 density range, while LpA-I/A-II predominates in HDL_3 density range (Cheung et al.1982). In addition, HDL can be distinguished into pre-α-HDL, α-HDL, and pre-β-HDL according to the particle surface charge (Davidson et al. 1994).

2.2.4.3. Metabolism of HDL particles

ApoA-I is secreted predominantly by the liver and small intestine as lipid-poor apoA-I and nascent discoidal, phospholipid-rich, cholesterol-poor HDL particles. The nascent HDL is also formed during lipolysis of TRLs, namely CMs and VLDLs (Eisenberg 1984). ApoA-II is produced in the liver only. The nascent lipid-poor apoA-I-phospholipid complexes are termed as pre-β-HDL particles due to their electrophoretic motility (Davidson et al. 1994). ATP-binding cassette transporter A1 (ABCA1) is an important cellular protein that facilitates efflux of cellular choles-

terol to the pre-β-HDL particles (Lawn et al. 1999). LCAT esterifies the cholesterol on the nascent HDL, transforming the discoid particles into mature, lipid-rich, and spherical HDL containing a core of cholesteryl esters and two molecules of apoA-I (Rye et al. 1999). The apolipoproteins in HDL, including apoA-I, apoA-II, apoA-IV, and apoC-I, activate the esterification of LCAT. PLTP, in turn, transfers the surface lipids from the post-lipolytic VLDL and CM particles to HDL (Tall 1995). The net effect of CETP action on HDL is depletion of cholesteryl esters and enrichment with TGs. Hepatic lipase converts TG-enriched HDL to smaller HDL remnants, pre-β-HDL and lipid-poor or free apoA-I (Clay et al. 1992). The endothelial lipase hydrolyses HDL phospholipids generating free fatty acids, which are taken up by the endothelial cells.

Lipids or proteins of mature HDL are removed from the circulation by at least two direct pathways, which involve the selective uptake of lipids by hepatic scavenger receptor class B type 1 and the holoparticle uptake by apoE or apoA-I receptors, respectively, and two indirect pathways, which involve the action of CETP and hepatic and endothelial lipases. The removal of lipids regenerates lipid-poor or lipid-free apoA-I, which can leave the plasma into the extravascular space. There they serve as acceptors of cellular lipids and thus again initiate the generation of HDL. In the kidney, these small particles are filtered and removed from the plasma (von Eckardstein et al. 2001) (Figure 4).

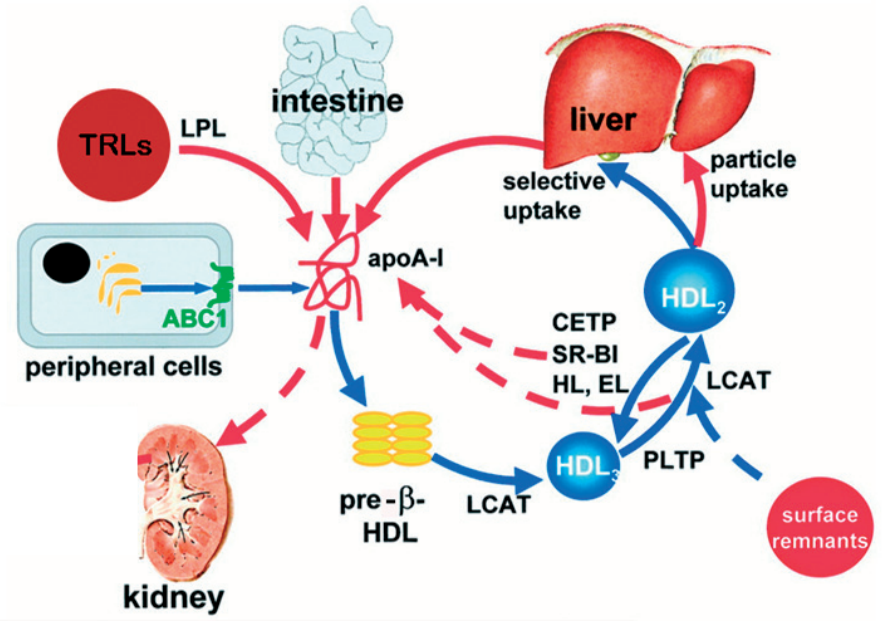


Figure 4. Pathways involved in the generation and conversion of HDL. For explanation, see text (chapter 2.2.4.). Blue arrows represent lipid transfer processes, and red arrows represent protein transfer processes. Abbreviations as in Figure 2.
Modified and used with permission from von Eckardstein et al. (2001) and the publisher (LWW).

2.3. Risk factors for atherosclerotic vascular disease

2.3.1. Definition of risk factor

Risk factors play an important role in initiating and accelerating the complex process of atherosclerosis. From an epidemiological perspective, a "risk factor" is a characteristic, a condition, or behavior of an individual or population that is associated with an increased risk for development of future disease. The definition is broad and does not necessarily imply a causal relationship. For a risk factor to be considered causal, the marker of interest must predate the onset of disease and must have biological plausibility. Therefore, much effort has been devoted to ascertaining whether various risk factors, particularly those that can be modified, are true causes of CVD.

Absolute risk refers to the probability of developing a condition (e.g., CAD) over a specific time period. Relative risk is the ratio of the absolute risk of a given patient (or group) to that of a low-risk group. The term relative risk represents the ratio of the incidence in the exposed population divided by the incidence in unexposed persons. In a sense, relative risk reflects the rate at which a person is accruing absolute risk (Grundy et al. 1998). Single risk factors, such as blood pressure or serum cholesterol are very poor predictors of absolute risk. Counting the number of risk factors present improves accuracy, but the most accurate method of absolute risk estimation is to count and weight appropriately all the major risk factors for CVD (Grover et al. 1995). This is done using risk equations, which are derived from large epidemiological studies.

2.3.2. Traditional risk factors

The Framingham Heart Study, initiated in 1948 in the predominantly Caucasoid general population of Framingham, Massachusetts, has contributed importantly to our understanding of the causes of coronary heart disease (CHD). The major and independent risk factors or the so-called traditional, established, or conventional cardiovascular risk factors, studied extensively and incorporated in the Framingham risk chart, are advancing age, gender, cigarette smoking, hypertension, elevated total and LDL cholesterols, low levels of HDL cholesterol, and DM. Family history has been omitted because its independent effect on CHD risk is less (Grundy et al. 1998). The Framingham risk chart provides an estimate of relative and absolute risk of developing CHD over a 10-year period.

Several problems have been noted with the Framingham risk chart, however. First, it was derived from American data, and the applicability of the risk chart to European population is uncertain. Second, the data set used for the chart was fairly small. Thirdly, the defintion of non-fatal end-points differed from those used in many other cohort studies, making it difficult to validate the chart (Conroy et al. 2003). Accordingly, a new model for total risk estimation based on the SCORE (Systematic Coronary Risk Evaluation) system has been launched. The SCORE risk assessment system is obtained from a large dataset of prospective European studies,

using five categorical variables, looking at northern and southern European countries, and measuring as the end point the absolute risk of cardiovascular mortality in the next 10 years. European guidelines recommend the SCORE as the new model for cardiovascular risk estimation (De Backer et al. 2003). Taken together, risk charts have been considered helpful in tailoring a plan for risk management in clinical practice.

2.3.2.1. Age

Although not subject to modification, age is among the most important risk factors for predicting incident CVD. The absolute risk for CVD increases with age in both men and women (Kannel et al. 1978, Schildkraut et al. 1989). In a 14-point scoring system, derived from the Framingham Heart Study, up to seven points can be attributed to age alone. Moreover, the risk of ischemic stroke doubles in each successive decade after 55 years of age (Wolf et al.1992). Thus, age is an overriding risk factor for incident CVD. It is acknowledged, however, that age is mainly a surrogate for an individual´s atherosclerotic burden, but a highly imprecise one (Grundy 1999). Replacement of age in risk assessment with a more precise marker – such as a non-invasive image modality – might improve predictive accuracy, but this hypothesis has not, as yet, been adequately tested.

At an individual level there is a wide variation in both the occurrence of CHD and the time of manifestation, even in individuals with much the same risk profiles. Therefore, the hypothesis has emerged that, at least to some extent, interindividual differences in biological ageing could affect susceptibility to CHD. Eukaryotic chromosomes end with telomeres, which progressively shorten with cellular ageing. Shorter telomeres indicate increased biological age. Interestingly, Brouilette et al. (2007) concluded, in a prospective randomised primary prevention trial of a statin, that individuals with shorter leucocyte telomere length at the time of recruitment had a significantly higher risk of developing subsequent CHD. Of note, this increased risk with shorter baseline telomeres was attenuated in subjects receiving treatment with a statin.

2.3.2.2. Gender

Epidemiological studies, including the Framingham Heart Study, have demonstrated that men exhibit excess risk for CVD compared with women, particularly premenopausal women (Kannel et al. 1995). This applies, however, only to the non-diabetic population, since DM largely blunts the benefit of female sex. The reasons for this gender difference in the impact of type 2 DM on CHD risk remain incompletely understood, but could be related at least in part to a heavier risk factor burden, a greater effect of blood pressure, and atherogenic dyslipidemia in diabetic women (Juutilainen et al. 2004).

The incidence of CHD in women lags behind that in men by 10 to 15 years, but the onset of menopause quadruples the risk of coronary events (Kannel et al.

1995). Some of this apparent protection could be due to the fact that premenopausal women exhibit relatively lower concentrations of total cholesterol, LDL cholesterol, and TGs and higher concentrations of HDL cholesterol than age-matched men (Williams 1997). Moreover, cigarette smoking has been substantially less frequent in the past in women compared to men, but trends appear to be reversing in young women (Collins 2006).

An impressive body of evidence from epidemiological studies has indicated a powerful protective effect of hormone-replacement therapy on the risk and development of CHD in postmenopausal women (Collins 2006). However, the results from both primary (Writing Group for the Women´s Health Initiative [WHI] investigators 2002) and secondary prevention trials (Hulley et al. 1998) have failed to show any benefit of hormone-replacement therapy on CHD risk. These divergent findings were suprising, especially in view of the positive impact of estrogen therapy on circulating LDL, HDL, and Lp(a) concentrations (Humphrey et al. 2002). The failure of the clinical trials to demonstrate a reduction in CHD events may be, in part, due to the selection of the population in terms of age and to the dose and possibly the type of steroids being employed. Notably, hormone-replacement therapy has also been associated with potentially unfavorable effects, including increased levels of TGs, factor VII, C-reactive protein, and decreased levels of antithrombin III (Humphrey et al. 2002).

2.3.2.3. Smoking

Cigarette smoking has long been recognized as a risk factor for CVD. Framingham data reveal that smoking is a powerful risk factor for myocardial infarction, even stronger than for angina pectoris (Hubert et al. 1982) and smokers have a 1.8-fold increase in stroke risk (Wolf et al. 1991).

The pathophysiological effects of smoking are multifactorial. Smoking causes reduced blood vessel distensibility and compliance, thus leading to increased wall stiffness (Kool et al. 1993). Moreover, smoking is associated with increased fibrinogen levels, increased platelet aggregation, decreased HDL cholesterol levels, and increased hematocrit (Cruickshank et al. 1989). Taken together, smoking probably destabilizes plaques and promotes plaque rupture and thrombosis.

The risk of future CVD is particularly high if smoking starts before the age of 15 years (Kawachi et al. 1993). Moreover, the adverse effect of smoking is related to the amount of tobacco smoked daily and to the duration of smoking (Teo et al. 2006). On the other hand, smoking cessation rapidly and markedly reduces the risk for myocardial infarction. A large part of the excess risk of myocardial infarction associated with smoking dissipates within five years, and among light smokers (<10 cigarettes per day) there is no excess risk 3-5 years after quitting. However, the excess risk does not seem to have completely disappeared even 20 years after quitting in those who are heavy smokers (≥20 cigarettes per day) (Teo et al. 2006).

2.3.2.4. *Hypertension*

The importance of elevated blood pressure, both systolic and diastolic, as a risk factor for CVD in both men and women has been demonstrated in a large number of epidemiological studies (Assmann et al. 1988, MacMahon et al. 1990, Kannel 1996). The approximately linear relation between blood pressure and the incidence of CVD is supported by numerous studies demonstrating that antihypertensive therapy does in fact decrease the risk for both heart attack and stroke (Moser et al. 1991, Cutler et al. 1995). However, the blood pressure elevation as well as its treatment is more obviously related to the incidence of stroke than to the incidence of CAD (MacMahon et al. 1990, Collins et al. 1992). It has also been documented that, compared with normotensive individuals, those with an elevated blood pressure more commonly have other risk factors (dyslipidemia, IR, DM) for CVD (Assmann et al. 1988, Isomaa et al. 2001).

2.3.2.5. *Total cholesterol and LDL cholesterol*

There is a strong and graded positive association between total as well as LDL cholesterol and the risk of CVD. The association applies to individuals with asymptomatic CVD as well as to patients with established disease (Neaton et al. 1992, Smith et al. 1992, Pedersen et al. 1998, Simes et al. 2002), to women as well as men, and to old as well as younger people (Clarke et al. 2002).

A recent prospective meta-analysis of data from 90 056 individuals in 14 randomised primary and secondary prevention trials showed that statin therapy can safely reduce the 5-year incidence of major coronary events, coronary revascularisation, and stroke by about one fifth per mmol/L reduction in LDL cholesterol, largely irrespective of the initial lipid profile (Cholesterol Treatment Trialists´ Collaborators 2005).

2.3.2.6. *HDL cholesterol*

One of the main CAD risk factors, discovered already in the early 1950s, is low HDL cholesterol (Nikkilä 1953). Thereafter, large prospective studies such as the Tromsø Heart-Study (Miller et al. 1977) and the Framingham Heart Study (Gordon et al. 1977, Abbott et al. 1988, Wilson et al. 1988) have confirmed the inverse association between HDL cholesterol levels and CAD. The estimated reduction of CAD risk by each 0.026 mmol/L (1 mg/dL) increase in HDL cholesterol is 2% in men and 3% in women (Gordon et al. 1989). The Copenhagen City Heart Study found a 47% reduction of ischemic stroke events for every 1-mmol/L increase in HDL cholesterol (Lindenstrom et al. 1994).

Despite a strong epidemiological association, the mechanism by which HDL exerts its beneficial effects on CHD is still debated. Traditionally, the main hypothesis for the anti-atherogenic function of HDL has been that of reverse cholesterol transport (Stein et al. 1999). Evidence that cholesterol efflux is indeed crucial has been provided in mice demonstrating that a selective expression of ABCA1 in mac-

rophages is anti-atherogenic, even though plasma lipoprotein levels remain unchanged (Aiello et al. 2002). Moreover, Attie et al. (2001) found, in heterozygotes for mutation in the ABCA1 gene, lower levels of apoA-I and HDL together with an increase of carotid IMT. Several alternative or concomitant hypotheses emphasizing the anti-atherogenic role of HDL have been presented including inhibition of LDL oxidation by HDL-bound PON1 (Mackness et al. 1993), protection of endothelial cells from cytotoxic damage caused by remnants of TRLs (Chung et al. 1989), regulation of coagulation and fibrinolysis and inhibition of platelet function (Nofer et al. 2002), stimulation of endothelial nitric oxide production (Yuhanna et al. 2001), and inhibition of the chemotaxis of monocytes and the adhesion of leukocytes to the endothelium (Nofer et al. 2002).

2.3.2.7. Diabetes mellitus

CAD causes much of the serious morbidity and mortality in patients with DM. This pertains to the more prevalent type 2 DM but also to type 1 DM of long duration, especially after the development of nephropathy. Diabetic patients have a 3- to 5-fold increase in the risk of CAD (Kannel et al. 1979, Pyörälä et al. 1987). Patients with medically treated DM but without previous myocardial infarction carry the same level of subsequent coronary events as non-diabetic patients with previous myocardial infarction (Haffner et al. 1998). Although in other populations pre-existing CAD has been a stronger risk factor for future CAD events than DM (Lotufo et al. 2001, Hu et al. 2001), the greatly increased risk conferred by DM is undisputable. In addition, the case-fatality rates of myocardial infarction are greater in diabetic compared with non-diabetic patients (Miettinen et al. 1998). These observations have led the Adult Treatment Panel III (ATPIII) of the National Cholesterol Education Program (NCEP) to establish type 2 DM as a CHD equivalent (Expert panel 2002). Further, individuals with DM have a 1.5- to 4-fold increase in the risk of ischemic stroke (Beckman et al. 2002).

Increasing evidence suggests that DM alters function of multiple cell types. Firstly, hyperglycemia impairs endothelium-dependent vasodilation by reducing ambient concentrations of nitric oxide. Concomitantly, vasoconstriction is augmented by increased production of endothelin-1 and angiotensin-II. Decreased nitric oxide and activation of receptors for advanced glycation end products enhance the activation of the transcription factors nuclear factor κB and activator protein 1. These factors induce the inflammatory gene expression, with liberation of leukocyte-attracting chemokines, increased production of inflammatory cytokines, and augmented expression of cellular adhesion molecules. Secondly, hyperglycemia activates protein kinase C, receptors for advanced glycation end products, and nuclear factor κB in vascular smooth muscle cells. Vascular smooth muscle cells are integral in the development of atherosclerosis by migrating into the nascent intimal lesion, replicating, and laying down a complex extracellular matrix. Thirdly, activation of protein kinase C and decreased production of platelet-derived nitric oxide also impairs platelet function (Beckman et al. 2002).

2.3.3. Beyond traditional risk factors

Framingham risk scores estimate risk for persons without manifest atherosclerotic disease. Therefore, the scores apply essentially to primary prevention, i.e. to prevention in persons without clinically established CAD. Once coronary atherosclerotic disease becomes clinically manifest, the risk for future coronary events regardless of other risk factors is much higher than that for patients without CAD. In this case, Framingham scoring no longer applies (Grundy et al. 1999).

Sudden death or nonfatal myocardial infarction occurs without prior recognized CAD symptoms in ≥25 % of patients (Myerburg et al. 1993). Approximately 40% of deaths from CAD occur in patients with total cholesterol levels lower than average for the general population (Smith 2006). Thus, in the last decade, the emphasis in CAD prevention has broadened from primary prevention based on single risk factors, such as hyperlipidaemia or hypertension, to the identification of higher-risk individuals on the basis of a global risk assessment (Smith et al. 2000, De Backer et al. 2003). Increasing research attention has been devoted to non-invasive methods for measuring subclinical atherosclerotic vascular disease (Greenland et al. 2001) and to more recently identified and less well-known factors.

2.3.3.1. Triglycerides

Despite more than 40 years of research the relation between TGs and CAD risk has been considered more or less enigmatic. A controversy has raged whether elevated TGs are an independent risk factor (Hulley et al. 1980) mainly due to biological and statistical problems. The measurement of total plasma TGs is hampered by considerable inter- and intraindividual variations (Bookstein et al. 1990). The plasma TG concentration increases and decreases throughout the day in response to food intake. TG levels vary considerably more than LDL and HDL cholesterol, with day-to-day variability being 23% for TGs, 9.5% for LDL, and 7% for HDL cholesterol (Smith et al. 1993). Furthermore, it has been shown, already in the 1970s, that TG levels are inversely correlated with HDL cholesterol levels (Schaefer et al. 1978). Usually, this strong metabolic interdependence thwarts attempts to assign statistically significant independence for these parameters. In fact, in multivariate analyses, low HDL cholesterol levels have been a more consistent and reliable predictor of increased CAD rates than elevated TG concentrations (Gordon et al. 1977, Abbott et al. 1988, Criqui et al. 1993, Wilson et al. 1994). Additionally, plasma TG is carried in a number of different lipoproteins, and the ability of different TRLs to promote atherosclerosis is not the same.

Although the impact of elevated TG levels on CAD risk has long been a matter of intense debate, a bulk of evidence supporting the prognostic value of this lipid constituent has been gained during the last decade (Cullen 2000). Recently, a meta-analysis including 262 525 participants and 10 158 CHD cases in 29 Western prospective studies found highly significant associations between TG values and CHD risk. However, adjustment for established coronary risk factors, especially HDL cholesterol, substantially attenuated the magnitude of this association (Sarwar et al. 2007).

The exact cellular links between elevated TGs and CAD are incompletely understood. Firstly, it is proposed that rather than being actual atherogenic agents themselves, elevated TGs merely serve as a marker for increases in remnants of TRLs and that it is these latter particles that are involved in the development of atherosclerosis (Havel 1990, Krauss 1998, Carmena et al. 2004). Secondly, hypertriglyceridemia has been associated with other atherogenic lipoprotein profiles, such as low HDL cholesterol and the presence of small, dense LDL particles. This "lipid triad" has also been termed atherogenic dyslipidemia commonly seen in patients with abdominal obesity, IR, and physical inactivity, all of which carry a markedly increased risk of CVD (Expert panel 2002). Thirdly, elevated TGs are associated with increased concentrations of factor VII and plasminogen activator inhibitor-1, which may accelerate the thrombotic processes (Krauss 1998).

2.3.3.2. Postprandial lipemia

2.3.3.2.1. Epidemiological and case-control studies

Zilversmit introduced postprandial lipemia as a putative atherogenic factor in 1979 (Zilversmit 1979). Since then growing evidence have indicated that postprandial TRLs play a role in the development of CAD. The mechanisms of atherogenicity have, however, remained poorly understood.

Several case-control studies, with mainly total TGs as postprandial lipid outcome variable, have suggested that patients with angiographically verified CAD have greater postprandial lipemia than control subjects (Simons et al. 1987, Simpson et al. 1990, Groot et al. 1991, Patsch et al. 1992, Syvänne et al. 1994a, Weintraub et al. 1996, Karpe et al. 1999). The magnitude of postprandial lipemia has also been related to the angiographic 5-year progression of CAD in young post-infarction patients (Karpe et al. 1994). Of note, there is a lack of results from prospective studies.

2.3.3.2.2. Determinants of postprandial lipemia

The magnitude of postprandial lipemia is closely correlated with fasting TG concentrations (Denborough 1963, Redgrave et al. 1979, Wilson et al. 1985, O´Meara et al. 1992). This mostly reflects the competition between elevated levels of endogenous VLDL and intestinally-derived CMs because both classes of particles share, at least partly, common removal mechanisms (Brunzell et al. 1973, Chen et al. 1991). Age appears to affect postprandial lipemia. Older subjects have higher postprandial TG levels than younger ones (Cohn et al. 1988, Krasinski et al. 1990). This could be due to prolonged residence time of CM remnants (Cohn et al. 1988) and to an increased VLDL production rate (Millar et al. 1995). Obesity, especially accumulation of visceral fat, is linked with postprandial lipemia even in normolipidemic subjects (Lewis et al. 1990, Couillard et al. 1998, Mekki et al. 1999). This may partly be related to differences in fasting TG concentrations between obese and lean subjects (Lewis et al. 1990). Regular exercise decreases postprandial lipemia (Nikkilä et al. 1962, Merril et al. 1989, Hartung et al. 1993).

2.3.3.2.3. Quantification of postprandial lipemia

Accurate quantification of remnant lipoproteins is problematic. Firstly, remnants are difficult to differentiate from their larger and more TG-rich precursors. Secondly, plasma concentration is low due to their rapid catabolism. Thirdly, they are biochemically difficult to isolate or detect because they are heterogeneous in size and composition (Cohn et al. 1999). To circumvent these problems, different methods for studying postprandial TRL metabolism have been employed.

The traditional method for isolating remnants has been ultracentrifugation allowing isolation of lipoproteins intermediate in density ($1.006<d<1.019$ g/mL, Sf 12-20) between VLDL and LDL. There is, however, no standardized clinical procedure for measurement of IDL concentration. Usually, IDL has been determined from simultaneously centrifuged sample of plasma, as the difference between cholesterol in the $d>1.006$ and $d>1.019$ g/mL fractions. However, this IDL fraction does not include larger, more TG-rich, less completely catabolized remnants (small VLDLs, Sf 20-60), which have also been shown to be atherogenic (Cohn et al. 1999)

Plasma lipoproteins have also routinely been separated according to their charge by agarose gel electrophoresis. Large TRLs or CMs remain at the origin of the gel, while ultracentrifugally isolated VLDLs ($d<1.006$ g/mL) normally migrate as a single band with pre-β mobility. Smaller, less TG-rich VLDLs are less negatively charged and migrate with slower mobility. TRL remnants are intermediate in size between VLDL and LDL and have been separated as "midband lipoproteins" by polyacrylamide tube or gradient gel electrophoresis. Several clinical studies on postprandial lipoprotein metabolism have utilized vitamin A to trace intestinal CM and their remnants. The rationale for this approach is that dietary vitamin A is incorporated into these particles as retinyl esters, mainly retinyl palmitate, which remain within the CM particle throughout its metabolism. This method is, however, not ideal because intestinal absorption of vitamin A is variable. Second, it does not allow assessment of the hepatic apoB-100 particles, and thirdly, retinyl esters can be detected in apoB-100 containing TRLs and in LDL and HDL at later postpranidal times (Cohn et al. 1999).

Although these techniques have provided important information on TRL remnants, they are complex, time consuming, and not optimal for use in clinical practice. Therefore, a more convenient method for isolation and quantification of remnant lipoproteins was developed in 1993 (Nakajima et al. 1993). In this assay, remnant lipoprotein particles (RLPs) are separated from plasma by immunoaffinity chromatography with a gel containing an anti-apoA-I and a specific apoB-100 monoclonal antibody. The former antibody recognizes all HDL and any newly synthesized CMs containing apoA-I, whereas the latter antibody recognizes all apoB-100-containing lipoproteins, except for certain particles enriched in apoE. Thus, HDLs, LDLs, large CMs, and the majority of VLDLs are retained by the gel. The unbound RLPs are made up of TRLs containing apoB-48 and a subfraction of apoB-100. RLPs are routinely measured in terms of the cholesterol content (RLP-C), although they can also be quantified in terms of TGs (RLP-TG) or specific apolipoproteins (Marcoux et al. 1998).

2.3.3.2.4. Determinants of remnant lipoprotein particles

Plasma levels of RLP-C in healthy, normolipidemic whites typically range from 0.16 to 0.24 mmol/L (6.2 to 9.3 mg/dL). RLP-C levels in Japanese subjects have consistently been reported to be lower than those in Caucasians. This may reflect ethnic differences or differences in the calibration of RLP-C assays in different laboratories. Higher levels of RLP-C have been demonstrated in men versus women, older versus younger, postmenopausal versus premenopausal women, the fed versus the fasted state, individuals with DM, and in patients with familial dyslipidemia (Twickler et al. 2004). Plasma concentration of RLP-C has shown to significantly correlate with the plasma concentration of total TG, VLDL triglyceride, and VLDL cholesterol, but not with LDL cholesterol (Cohn et al. 1999).

2.3.3.2.5. Remnant lipoprotein particles and CAD

Several epidemiologic studies have shown associations between RLP elevations and CAD (Devaraj et al. 1998, Takeichi et al. 1999, Kugiyama et al. 1999, Masuoka et al. 2000, McNamara et al. 2001, Fukushima et al. 2001). In 135 Japanese patients with CAD (Kugiyama et al. 1999), elevated RLP levels were an independent predictor of future coronary events. Two angiographic studies (Devaraj et al. 1998, Masuoka et al. 2000) have revealed that patients with significant lesions have higher RLP-C levels than patients without significant lesions. In contrast, in the first long-term prospective investigation recently conducted in a cohort of 1156 Japanese-American men from the Honolulu Heart Program, RLP levels did not provide additional information about CHD incidence as compared with concentration of total TGs. This could be attributed to the strong correlation between RLP levels and total TGs (Imke et al. 2005).

2.3.3.3. LDL particle size

Small, dense LDL particles bind less avidly to the LDL receptor than large, buoyant LDL resulting in decreased hepatic clearance and a longer residence time in plasma. The filtration rate of LDL particles into the arterial intima is inversely related to particle size. Therefore, small, dense LDL particles enter the arterial wall more easily (Chapman et al. 1998). Moreover, small, dense LDL particles show greater susceptibility to oxidation than large LDL (de Graaf et al. 1991, Tribble et al. 2001). Together, these findings have led to the hypothesis that small, dense LDL is a potent atherogenic lipoprotein.

Several lines of evidence obtained from both cross-sectional and prospective studies suggest that the predominance of small, dense LDL is associated with an increased risk of CAD (Rizzo et al. 2006). This has indeed been shown in the vast majority of univariate analyses, but after multivariate adjustment for confounding variables, in particular TGs and HDL cholesterol, LDL particle size is seldom a significant and independent predictor of CAD risk (Rizzo et al. 2006). Hence, it has been argued that LDL subclass measurement does not add information beyond measuring TG levels and LDL and HDL concentrations (Sacks et al. 2003).

The small, dense LDL phenotype rarely occurs as an isolated disorder. It is frequently accompanied by hypertriglyceridemia and low HDL cholesterol. These three abnormalities are metabolically intertwined and thus, it is not easy to single out small, dense LDLs as independent risk factors for CAD (Lada et al. 2004). Of note, there is only one study to date, which has demonstrated a shift in LDL particle size towards smaller, densier species during alimentary lipemia in patients with CAD (Koba et al. 2005).

Interestingly, recent studies have shown that the overall number of LDL particles in plasma, not their size, may provide a better indication of the risk of CAD (Lada et al. 2004). The number of LDL particles in plasma is potentially important, because the arterial walls are exposed to these particles, and an increased number might increase atherogenicity independently of particle size (Rizzo et al. 2006).

2.3.3.4. Oxidized LDL

2.3.3.4.1. Origin of the oxidative modification hypothesis

The oxidative modification hypothesis of atherosclerosis was proposed in 1989 (Steinberg et al. 1989). According to this hypothesis, oxidative modification of LDL is necessary and possibly obligatory in the development of atherosclerotic lesions. The original interest stemmed from seminal observations that native LDL was not taken up by macrophages rapidly enough to generate foam cells. It was postulated that circulating LDL must undergo some kind of structural modification before it becomes proatherogenic. Further, it was proposed that, the uptake of LDL into macrophages occurs through "scavenger" receptors rather than the classic LDL receptor (Goldstein et al. 1979). Since then, multiple studies in experimental animal models have provided firm evidence of an important role of oxLDL in atherogenesis (Berliner et al. 1996, Steinberg 1997, Chisolm et al. 2000). However, the exact in vivo mechanisms of LDL oxidation in humans are still unknown.

2.3.3.4.2. Biological activities of oxLDL

OxLDL has a wide range of atherogenic properties, from early lesion formation to plaque rupture. It contributes to atherogenesis by 1) inducing endothelium to express adhesion molecules for monocytes, intercellular adhesion molecule-1, and vascular adhesion molecule-1, 2) increasing monocyte chemotaxis and adhesion, 3) compromising endothelial function through inhibition or reduced production of nitric oxide, 4) upregulating inflammatory genes and growth factors, and 5) stimulating growth of smooth muscle cells, platelet aggregation, and thrombus formation (Tsimikas et al. 2001). OxLDL also stimulates an immunological response seen as autoantibodies against several epitopes of oxLDL (Chisolm et al. 2000). Finally, the uptake of oxLDL via scavenger receptors leads to massive deposition of lipids into the macrophages, i.e. the formation of foam cells. Foam cells form the core of the earliest atherosclerotic lesion, the fatty streak.

There is substantial evidence that oxLDL is present in vivo within atherosclerotic but not normal blood vessels (Ylä-Herttuala et al. 1989, Witztum et al. 2001). LDL that is extracted from human and animal atherosclerotic lesions has all of the physical, chemical, immunological, and biological properties of oxLDL. Monoclonal antibodies to epitopes of oxLDL immunostain animal and human atherosclerotic lesions. Autoantibodies against oxLDL, reflecting the fact that oxLDL is very immunogenic, have been found in lesions and plasma of animals and patients with various manifestations of atherosclerosis (Tsimikas et al. 2001).

2.3.3.4.3. Measurement of oxLDL

Until recently, methods for direct measurement of oxLDL in blood were lacking. Initially, an assay measuring the level (the titer) of autoantibodies against neoepitopes in LDL oxidized by copper or modified by malondialdehyde, a highly reactive breakdown product formed during peroxidation of polyunsaturated fatty acids in phospholipids and cholesteryl esters, was developed as an indirect indication of in vivo oxidation of LDL. Autoantibodies against oxLDL have been reported to be associated with coronary atherosclerosis. However, the data are not consistent (van de Vijver et al. 1996, Uusitupa et al. 1996, Orchard et al. 1999, Lehtimäki et al. 1999), possibly due to assay divergencies in the different studies (Mertens et al. 2001).

Nowadays, more sensitive enzyme-linked immunosorbent assay (ELISA) methods to "directly" measure the small amount of circulating oxLDL are available. In these assays, different monoclonal antibodies against a neo-epitope in the aldehyde-substituted apoB-100 moiety of oxLDL (Holvoet et al. 1998) or against an epitope in the oxidized phospahtidylcholine (Itabe et al. 1996) are used to recognize a single epitope in the circulating oxLDL.

2.3.3.4.4. OxLDL in cardiovascular disease

To date, a number of studies in the cardiovascular field have measured oxLDL levels by utilizing competitive ELISA. Holvoet et al. (1998) found elevated plasma concentrations of oxLDL in patients with stable CAD and in subjects with acute coronary syndromes compared with age-matched control subjects. In a Japanese case-control study (Toshima et al. 2000), the concentrations of oxLDL were 1.9-fold higher in patients with angiographically proven CAD than in healthy controls. Furthermore, Holvoet et al. (2001) demonstrated, in 178 patients with angiographically verified CAD and 126 age-matched subjects, that patients with CAD had significantly elevated plasma levels of oxLDL.

Elevated levels of oxLDL in vivo have also been found in patients with impaired glucose tolerance (Kopprash et al. 2002). Notably, it has been shown in type 2 DM that postprandial LDLs are oxidized more extensively in vitro than fasting diabetes and control samples (Diwadkar et al. 1999). Recently, Meisinger et al. (2005) concluded, in a prospective population study comprising men, that oxLDL was a stronger predictor of risk than standard lipid variables and other traditional CAD risk factors. Further, Holvoet et al. (2004) found, in a population-based cohort of

3033 individuals aged 70-79 years at baseline, that the metabolic syndrome was associated with higher levels of oxLDL. However, oxLDL was not an independent predictor of total CHD risk.

2.3.3.5. Paraoxonase-1 (PON1)

A large body of evidence has accrued indicating that the antioxidant property of HDL is largely mediated by the HDL-bound enzyme PON1 (Durrington et al. 2001). PON1 was initially studied, more than 50 years ago, in the field of toxicology because of its ability to hydrolyze, and hence detoxify, organophosphates. The name PON reflects its ability to hydrolyze paraoxon, a toxic metabolite of the insecticide parathion. Parathion is toxic to humans because of its irreversible inhibition of acetyl cholinesterase (Aldrige 1953a, 1953b). In addition, paraoxonase hydrolyzes active metabolites of a number of other organophosphorus insecticides (e.g., chlorpyrifos oxon, dizoxon) as well as nerve agents such as sarin and soman (Costa et al. 2005).

2.3.3.5.1. Synthesis and structure of PON-1

The human genome contains at least three PON genes, designated PON1, PON2, and PON3. All three genes reside on the long arm of human chromosome 7. The genes share about 65% similarity at the amino acid level and approximately 70% similarity at the nucleotide level (Primo-Parmo et al. 1996). Human PON1, so far the most studied of the three known PONs, is a glycoprotein comprising 354 amino acids with a molecular mass of about 44 kDa (Furlong et al. 1993).

In humans, PON1 mRNA expression is mainly limited to the liver, while PON3 mRNA is expressed in the liver and kidney, and PON2 is widely expressed in most tissues including the heart, kidney, liver, lung, placenta, small intestine, spleen, stomach, and testis. Furthermore, unlike PON1 and PON3, PON2 is also detected in the cells of the artery wall, including endothelial cells, smooth muscle cells, and macrophages. PON1 and PON3 specifically associate with HDL in circulation, whereas PON2 is not linked to HDL (Primo-Parmo et al. 1996, Ng et al. 2005). Recently, PON1 was also shown to be associated with VLDL, but not with LDL (Deakin et al. 2005). Both human PON2 and PON3 possess antioxidant properties, similar to those of PON1, and are capable of preventing oxidative modification of LDL (Ng et al. 2005).

2.3.3.5.2. PON-1 gene

PON1 contains several polymorphic sites, i.e. two or more variants (alleles) exist at significant frequencies in the population. One specific form of polymorphism is the single nucleotide polymorphism defined as a specific difference in one base at a defined location of an individual´s DNA. PON1 has two single nucleotide polymorphisms in the coding region of the gene that change the amino acid of the protein: a methionine (M) to leucine (L) substitution at position 55 (M/L55) and a glutamine

(G) to arginine (A) substitution at position 192 (Q/R192). In addition to these two coding polymorphisms, at least five polymorphisms have been detected in the PON1 promoter region (Ng et al. 2005).

PON1 activity can vary up to 40-fold in human populations (Mueller et al. 1983, Richter et al. 1999). Part of this variability is explained by the Q/R192 polymorphism (Adkins et al. 1993). This Q/R192 polymorphism has been more widely studied, because the two alloenzymes have different affinities and catalytic activities towards a number of substrates. The R-allele at position 192 displays several-fold higher activity towards paraoxon hydrolysis, whereas the Q-allele is more active towards sarin, soman, and diazoxon (Aviram et al.1998a). In contrast, phenylacetate hydrolysis is largely unaffected by the polymorphism, and has been used as a surrogate for protein concentrations (Cao et al. 1999).

2.3.3.5.3. Measurement of PON-1 activity and concentration

PON1 has two qualitatively different properties, paraoxonase and arylesterase activities, because it hydrolyzes both organophosphates and aromatic esters (Mackness et al. 1987). Paraoxonase activity is measured from serum samples by hydrolysis of paraoxon to the non-toxic products, p-nitrophenol and diethyl phosphate (Aldridge 1953, Erdös et al. 1961). The activity is first measured without any added NaCl (basal activity) and then with 1 M NaCl included. The percent stimulation of paraoxonase by 1 M NaCl is used to classify individuals into the non-salt-stimulated A type, and salt-stimulated AB and B types (Eckerson et al. 1983a), since measurement of paraoxonase activity alone does not clearly discriminate between AB and B phenotypes (Flugel et al. 1978).

Arylesterase activity is usually measured with phenylacetate as a substrate (Mounter et al. 1953, Simpson 1971). The distribution of arylesterase activity in a Caucasian population has a single mode while the paraoxonase activity is bimodally distributed. The distribution of the ratio of salt-stimulated paraoxonase activity to arylesterase activity, however, is clearly trimodal indicating the existence of three paraoxonase phenotypes (Eckerson 1983b). The serum PON1 concentration can be determined by a competitive ELISA using a monoclonal antibody (Blatter Garin et al. 1994) or by sandwich ELISA using two monoclonal antibodies (Kujiraoka et al. 2000).

2.3.3.5.4. Antioxidant properties of PON-1

In vitro studies have shown the ability of PON1 to block the accumulation of lipid peroxides in LDL (Mackness et al. 1993, Watson et al. 1995). Several studies have confirmed and extended this finding, demonstrating that PON1 prevents the formation of oxLDL and inactivates LDL derived oxidized phospholipids once they are formed. PON1 also protects phospholipids in HDL from oxidation (Costa et al. 2005). The most convincing data to link PON1 with CAD come from animal studies. In knockout mice lacking the gene for PON1, atherosclerosis develops more rapidly than in wild-type mice, whereas mice that overexpress human PON1 are resistant to atherosclerosis (Shih et al. 1998, Tward et al. 2002).

2.3.3.5.5. PON-1 genotype and CAD

Hypothesized differences in the ability of the polymorphic forms to protect oxidation of LDL have led to numerous studies attempting to determine the relationship between PON1 polymorphisms and CAD. The majority has reported on the PON1 Q/R192 polymorphism, fewer studies have been conducted on the PON1 M/L55 polymorphism. The results, no matter what the polymorphism investigated, have proved contradictory (Durrington et al. 2001, Wheeler et al. 2004). A recent meta-analysis using all 43 available studies on the PON1 polymorphisms involving 11212 CAD cases and 12786 controls suggested that the link between PON1 Q/R192 polymorphism and CAD was at best weak (Wheeler et al. 2004). There were no significant associations between PON1 M/L55 polymorphism and CAD. Mostly, CAD was defined as a history of myocardial infarction and/or significant (≥50% of the luminal diameter) angiographic coronary stenosis. Quantitative coronary angiography (QCA) was utilized in two studies (Turban et al. 2001, Chen et al. 2003a). In a report from the Women´s Ischemia Syndrome Evaluation (WISE) study including 711 women, Chen et al. (2003a) did not find any significant association between the PON polymorphisms and stenosis severity in either white or black women. However, when patients with significant CAD (≥50% stenosis) were stratified into groups with one-, two-, or three-vessel CAD, significant associations were noted between PON polymorphisms and the number of diseased vessels in white but not in black subjects. In the study by Turban et al. (2001), Q/R192 polymorphism was not associated with the progression of coronary atherosclerosis determined by serial QCA. Of note, this was the only prospective trial reported in the meta-analysis.

2.3.3.5.6. PON-1 activity and concentration and CAD

Investigating only an association between PON1 polymorphism and vascular disease may not adequately assess the protective effect of the enzyme, which has led to the proposal that PON1 activity and concentration rather than genotype alone may be more important in determining atherosclerosis risk (Mackness et al. 2001). Very few studies have included a measure of PON1 activity and concentration. Mackness et al. (2001) found in a case-control study, that PON1 activity toward paraoxon and PON1 concentration were lower in 417 subjects with angiographically proven CAD regardless of PON1 genotype. Similarly, although the difference did not reach statistical significance, Azarisiz et al. (2003) found that PON1 activity of patients with CAD (n=68) was lower than that of patients without CAD (n=33) and control subjects (n=24). In addition, there is one prospective epidemiologic study, which has shown in middle-aged men that low PON1 activity toward paraoxon is an independent risk factor for coronary events (Mackness et al. 2003). However, PON1 concentration was not related to new CAD events. The reason why only paraoxon hydrolytic capacity reflected PON1 as a risk factor for CAD was unclear.

2.3.3.6. Apolipoprotein B

ApoB-100 is the major apolipoprotein component of the atherogenic lipoproteins VLDL, IDL, LDL, and Lp(a). ApoB has been proposed (Sniderman et al. 1996) and debated (Rader et al. 1994) as risk factor for CAD. Some studies, but not all, have suggested that the measurement of apoB in plasma may provide more information on CAD risk than LDL cholesterol alone (Durrington et al. 1986, Stampfer et al. 1991, Rader et al. 1994). Some of these discrepancies may have resulted, in part, from difficulties in standardizing assays for measuring plasma apoB levels. However, standardized methods have been developed (Marcovina et al. 1994) and population results have subsequently been reported. In fact, four large, prospective epidemiologic studies (Quebec Cardiovascular Study [Lamarche et al. 1996], Moss Heart Study [Moss et al. 1999], AMORIS Study [Walldius et al. 2001], Northwick Park Heart Study [Talmud et al. 2002]) have shown that apoB is superior to total or LDL cholesterols. In addition, the Quebec Cardiovascular Study demonstrated increased risk associated with small, dense LDL, while the Northwick Park Heart Study identified TG and apoB as the most informative pair of lipid risk factors. The Apolipoprotein-related Mortality Risk (AMORIS) Study, in which 98722 men and 76831 women were followed for an average of 67 months, LDL cholesterol was predictive in men, but not in women. Further, LDL cholesterol was predictive of risk in those <70 years, but it was not predictive in those >70 years of age. By contrast, apoB was predictive in both men and women, and both above and below the age of 70. The AMORIS Study suggests that apoB should be regarded as highly predictive in evaluating cardiac risk. Further, apoB might be of greatest value in the diagnosis and treatment of persons with normal or low concentrations of LDL cholesterol.

It has recently been proposed that apoB should be included in all guidelines as an indicator of cardiovascular risk (Barter et al. 2006). Standardized apoB measures are not currently widely available in clinical practice. However, non-HDL cholesterol has shown to be significantly correlated with apoB and can represent an acceptable surrogate marker (Vega et al. 1990, Abate et al. 1993). Non-HDL cholesterol, routinely calculated as total cholesterol minus HDL cholesterol, is the sum of VLDL + IDL + LDL cholesterols and thus, an indirect measure of atherogenic lipoproteins containing apoB.

2.3.3.7. Apolipoprotein E

2.3.3.7.1. Structure and function of apoE

ApoE is a 299 amino-acid protein that is synthesized and secreted from hepatocytes and macrophages. The main physiological role of apoE is to mediate the interaction of lipoproteins with their receptors, including the LDL receptor and the LDL receptor-related protein. Since it serves as a ligand for these receptors, apoE plays a critical role in determining the clearance of remnants of CM and VLDL (Davignon et al. 1999, Curtiss et al. 2000). In vitro studies have shown that apoE modulates

activities of lipoprotein and hepatic lipases (Wang et al. 1985). It has also been demonstrated that heparan sulfate proteoglycans facilitate the uptake of atherogenic lipoproteins by ligand transfer to LDL receptor-related protein and that apoE can mediate the seuquestration or trapping of the remnants by binding to heparan sulfate proteoglycan (Mahley et al. 1995). In addition, apoE can promote the cholesterol efflux from macrophages, thus facilitating the reverse cholesterol transport from peripheral tissues to liver (Lin et al. 1999).

The three common apoE isoforms, designated E2, E3, and E4, are encoded for by distinct alleles on human chromosome 19. They are distributed in six different phenotypes ranking from most to least common E3/E3, E4/E3, E3/E2, E4/E4, E4/E2, and E2/E2 (Davignon et al. 1999). ApoE polymorphism is estimated to explain 4-15% of the variation in LDL concentrations. Compared to apoE3 or apoE2 isoforms, the presence of apoE4 is associated with higher levels of LDL cholesterol, TGs, and apoB, and lower concentrations of HDL cholesterol (Davignon et al. 1999, Curtiss et al. 2000). The mechanisms that are responsible for such changes are currently poorly understood except for the variation in cholesterol levels, where an action on hepatic LDL receptor levels is likely to make a major contribution (Mahley et al. 2000). Functionally, apoE2 has strikingly reduced affinity in vitro for the LDL receptor and moderately reduced affinity for LDL receptor-related protein, whereas apoE4 has somewhat increased affinity for the LDL receptor and comparable affinity for the LDL receptor-related protein. ApoE4 preferentially distributes to TRLs and accelerates it uptake, thereby leading to down-regulation of hepatic LDL receptor expression and increased levels of LDL (Davignon et al. 1999, Mahley et al. 2000). The concentration of apoE has been reported to be lower in apoE4 carriers than in those without apoE4 (Smit et al. 1988) suggesting that differencies in apoE concentrations could determine atherogenity linked to apoE.

ApoE knockout mice are especially prone to atherosclerosis (Davignon et al. 1999). Interestingly, physiological levels of apoE in mice have recently been shown to induce atherosclerosis regression independently of lowering plasma cholesterol levels (Raffai et al. 2005).

2.3.3.7.2. ApoE polymorphism and CAD

Numerous studies have investigated the relationship between apoE polymorphism and CAD, often yielding conflicting results. Following the observation that the presence of apoE4 is associated with higher LDL cholesterol levels, several studies, but not all, link the ε4 allele with greater risk for cardiovascular diseases (Eichner et al. 2002, Kolovou et al. 2003, Song et al. 2004). For example, a study comprising middle-aged men from nine populations estimated a 40% greater risk for mortality from CAD for carriers of the ε4 allele than ε2 carriers or individuals with the ε3/ε3 genotype (Stengard et al. 1998). In contrast, data from a prospective study including 385 incident cases and 373 controls did not support the simple view of E2 as a protective factor and E4 as a susceptibility factor in predicting future risk of myocardial infarction (Liu et al. 2003). Nevertheless, a comprehensive recent meta-analysis

of 48 published studies, comprising 15492 case patients and 32965 controls, concluded that carriers of the ε4 allele had a 42% higher risk for CHD compared with individuals with the ε3/ε3 genotype. The ε2 allele had no significant association with CHD risk (Song et al. 2004).

2.3.3.8. Lipoprotein(a)

2.3.3.8.1. Structure and function of Lp(a)

Lp(a), first described in 1963 (Berg 1963), is a complex genetic variant of LDL in which apoB 100 is linked by a disulfide bond to apo(a). Apo(a) is a highly glycosylated protein displaying size heterogeneity and molecular mass varying between 300 and 800 kDa. The apo(a) gene has been localized to the long arm of chromosome 6 (q26-27) where it is closely linked to the plasminogen gene. The concentration of Lp(a) shows considerable inter- and intraindividual biological variation. The consequent inherited differences in apo(a) molecular mass are largely responsible for the wide range of Lp(a) concentrations in different individuals with low levels predominating in European populations. At least 34 phenotypes are expressed, and there are potentially more phenotypes and genotypes that may exist. The apoE phenotype has also been suggested to play part in determining Lp(a) concentrations, the lowest levels being associated with the apoE2 phenotype and the highest with the apoE4 phenotype. In addition, Lp(a) levels can be modified by disease, particularly renal disease. Chronic renal failure or proteinuria, even with relatively well preserved creatinine clearance, both increase the median Lp(a) level on average by three to four times that of matched healthy controls. Lp(a) is associated with atherosclerosis in chronic renal failure (Durrington 1995). The similarity of Lp(a) to LDL and plasminogen has generated considerable interest, and proposed mechanisms of accelerated atherothrombosis caused by high levels of Lp(a) are shown in Table 2.

Table 2. Mechanisms of accelerated atherothrombosis caused by high levels of Lp(a).

Is readily oxidized and forms highly atherogenic particles with LDL
Enhances oxidation, uptake, and retention of LDL
Promotes lipid uptake by macrophages
Enhances expression of intercellular adhesion molecules
Increases smooth muscle cell proliferation and migration
Competes with plasminogen for binding to receptors in platelets, fibrin, endothelial- and mononuclear cells
Inhibits tissue plaminogen activator and enhances production of plasminogen activator inhibitor
Increases formation of thrombin
Decreases plasmin formation and impairs fibrinolysis

Adapted from Enas et al. (2006).

2.3.3.8.2. Lp(a) and CAD

Among numerous retrospective case-control studies, virtually all have shown an association between elevated Lp(a) levels and CAD (Marcovina et al. 1998). In contrast, several prospective studies have yielded apparently conflicting results, ranging from a strongly positive association to no association at all (Marcovina et al. 1998). Nevertheless, a meta-analysis (Danesh et al. 2000), including 27 prospective studies with information on 5436 CAD cases observed during mean follow-up of 10 years, supported an independent predictive power for elevated Lp(a). These prospective studies of CAD generally reported little or no change in risk ratios after adjustment for other risk factors. This suggests that any effects of Lp(a) are unlikely to be accounted for by effects on, or confounding with, classical vascular risk factors (Danesh et al. 2000). Further, the Prospective Epidemiological Study of Myocardial Infarction (PRIME), a recently conducted cohort study comprising 9133 men from France and Northern Ireland with no history of CHD or use of hypolipidemic drugs, confirmed Lp(a) as a predictor of CHD risk (Luc et al. 2002).

2.3.3.9. Insulin resistance

2.3.3.9.1. Overview

IR, characterized by a diminished response to the biological effects of insulin, is a strong predictor of type 2 DM (Lillioja et al. 1993). In addition, IR has been associated with obesity, predominantly intra-abdominal distribution of fat, hypertension, low HDL cholesterol, and hypertriglyceridemia, all of which are well-known risk factors for CAD (Reaven 1988, DeFronzo et al. 1991).

2.3.3.9.2. Assessment of IR – the homeostasis model assessment

The euglycemic hyperinsulinemic clamp technique has been regarded as the reference method for evaluating IR. However, this method is expensive, technically complex, and therefore unsuitable in clinical practice. In comparison with the euglycemic clamp, the homeostasis model assessment (HOMA) is an easy, practical, and inexpensive method for assessing IR. The HOMA is a method for assessing β-cell function and/or IR from the basal concentrations of glucose and insulin using a simple mathematical calculation (Matthews et al. 1985). The HOMA model has been shown to correlate moderately well with insulin sensitivity as measured using the euglycemic clamp technique. Already quoted in over 500 publications, the HOMA model has become a widely used tool in clinical and epidemiological research (Wallace et al. 2004).

HOMA IR, reflecting both peripheral and hepatic insulin sensitivity, is based upon the correlation between insulin and glucose values and the assumption that rising glucose concentrations lead to a compensatory increase in insulin concentrations. Thus, HOMA IR is highly dependent on the fasting insulin level and it has been argued that calculating HOMA IR in normal population is no better than

measuring fasting insulin concentrations. Furthermore, it has been reported that the intraindividual variation in HOMA-derived IR is much greater in subjects with type 2 DM than in non-diabetic subjects (Jayagopal et al. 2002). The reasons for this high inherent variability may be due to the underlying biological variability in insulin levels, arising from the combination of its short half-life, the known cyclicity of insulin secretion (Matthews et al. 1983), and the rapid responsiveness to changes in hormonal and metabolic milieu.

2.3.3.9.3. IR and development of cardiovascular disease

Given that IR is related to cardiovascular risk factors, it seems reasonable to believe that IR should be strongly related to cardiovascular disease. Surprisingly, there has been a marked controversy about this issue (Stern 1996). Hyperinsulinemia has been identified as a risk factor for CAD in several (Després et al. 1996, Pyörälä et al. 1998, Hanley et al. 2002), but not in all prospective studies (Welin et al. 1992, Ferrara et al. 1994, Orchard et al. 1994, Resnick et al. 2003). The Helsinki Policemen Study showed that hyperinsulinemia was associated with an increased CHD risk over a 22-year follow-up in men (Pyörälä et al. 1998). In the San Antonio Heart Study, where a population cohort was studied between 1984-1988 and followed up 7 years later, IR as calculated by the HOMA was associated with an increased CVD incidence independently of several cardiovascular covariates (Hanley et al. 2002). In contrast, in a study among non-diabetic American Indians, IR estimated by HOMA predicted DM, but did not predict CVD independently of other risk factor (Resnick et al. 2003). Further, the negative studies by Welin et al. (1992) and Ferrara et al. (1994) were done in elderly subjects and thus could have represented a survival bias. The study by Orchard et al. (1994) was done in high-risk subjects and thus its study population might have been enriched in subjects with IR.

2.3.3.9.4. IR and coronary atherosclerosis

Results from cross-sectional studies about the association between IR and the angiographic characteristics of coronary atherosclerosis estimated by conventional coronary angiography have yielded apparentely conflicting results (Bressler et al. 1996, Sasso et al. 2004, Yanase et al. 2004, Satoh et al. 2005). In a small study comprising 13 subjects with normal glucose tolerance, IR, as measured by the clamp technique, was positively correlated with the severity of CAD (Bressler et al. 1996). Sasso et al. (2004) showed that, in 234 men with normal glucose tolerance, the number of stenosed coronary vessels was correlated with HOMA IR. Moreover, Yanase and colleagues (2004) reported that HOMA IR was an independent risk factor of a new cardiovascular event in 102 patients with prior CAD and normal glucose tolerance. In contrast, Satoh et al. (2005) did not find a significant relationship between HOMA IR and the number of diseased coronary arteries, although post-challenge hyperinsulinemia showed a relation with the severity of CAD.

2.3.3.9.5. Atherogenic properties of IR

Data suggest that high amounts of fat tissue and derangements of the adipose tissue metabolism are the primary factors predicting the development of IR (Ruan et al. 2004). Adipose tissue is a complex and highly active metabolic and endocrine organ. Several adipocyte-derived proteins, such as tumor necrosis factor α, interleukin-6, tissue factor, and plasminogen activator inhibitor-1, have been implicated in the pathogenesis of obesity and IR (Kershaw et al. 2004). Adiponectin is the only adipocyte-derived hormone that is known to increase insulin sensitivity in the liver and skeletal muscle (Kadowaki et al. 2005). Kadowaki et al. (2005) showed that genetic factors in the adiponectin gene itself and environmental factors causing obesity such as a high-fat diet reduced adiponectin levels. They proposed that reduction of adiponectin might play a crucial causal role in the development of IR, type 2 DM, and atherosclerosis. Indeed, a low level of adiponectin has been shown to be associated with the prevalence of CAD, and to predict the occurrence of myocardial infarction (Nakamura et al. 2004, Pischon et al. 2004). Interestingly, Mazurek et al. (2003) demonstrated that epicardial adipose tissue in 55 patients undergoing elective coronary artery bypass grafting, exhibited significantly higher levels of chemokines (monocyte chemotactic protein-1) and several inflammatory cytokines (interleukin-6, interleukin-1β, and tumor necrosis factor α) than subcutaneous fat. In addition, the presence of inflammatory mediators in the tissues surrounding epicardial coronary arteries was noted irrespective of DM, obesity, or chronic therapy with statins.

The endothelium plays a vital role in vascular tone regulation through the release of both powerful vasodilating (e.g., nitric oxide) and vasoconstricting (e.g., endothelin-1) substances (Yanagisawa et al. 1988). In healthy subjects insulin is known to have a direct vasodilatory effect by stimulating endothelial production of nitric oxide (Kuboki et al. 2000). In insulin-resistant individuals, however, the ability of insulin to stimulate production of nitric oxide is diminished. Moreover, chronic exposure to insulin causes coronary vasoconstriction by increasing the release of endothelin-1 and sympathetic nerve activity (Sundell et al. 2003).

Endothelial dysfunction, an imbalance between endothelium-derived vasodilative and vasoconstrictive factors, is regarded as an early pivotal event in atherogenesis and cardiovascular disease and is closely linked to obesity and IR (Steinberg et al. 1996). In addition, obesity, IR, and endothelial dysfunction coexist and they can all be identified in individuals with type 2 DM as well as in various groups at risk for type 2 DM, such as individuals with impaired glucose tolerance, family history of type 2 DM, hypertension, and dyslipidemia (Caballero et al. 2003). Recently, Prior and co-workers (2005) demonstrated that, even in the absence of traditional coronary risk factors, the greatest loss in nitric oxide-mediated, endothelium-dependent flow occurred when IR was the only abnormality, and this seemed to worsen progressively with more severe states of IR.

2.4. Quantification of atherosclerosis

2.4.1. Coronary angiography

First performed by Sones in the late 1950s (Sones 1959), coronary angiography has subsequently become one of the most widely used invasive procedures in cardiovascular medicine. Although angiographic and imaging techniques have advanced over the past four decades, the analysis of coronary angiograms, by visually estimated percent diameter stenosis (PDS), has remained unchanged in most clinical catheterization laboratories.

Coronary angiography is used to establish the presence or absence of coronary stenosis, define therapeutic options, and determine prognosis in patients with symptoms or signs of ischemic CAD. The limitations of coronary angiography in the evaluation of the severity and extent of CAD are well recognized (de Feyter et al. 1991). An angiographic image is a two-dimensional shadowgram of an opacified vessel lumen providing only a measure of residual lumen or relative stenosis. However, atherosclerotic changes in the arterial wall are not reliably or precisely reflected by changes in the lumen. Necropsy studies demonstrate that CAD is frequently diffuse and contains no truly normal segment (Schwartz et al. 1975, Hutchins et al. 1977, Arnett et al. 1979). In the presence of diffuse disease, calculation of the angiographic percent stenosis will predictably underestimate disease severity.

Angiography is often confounded by the phenomenon of arterial remodeling. Compensatory enlargement of the vessel wall results in preservation of nearly normal lumen cross-sectional area so that angiography severely underestimates or is unable to detect early stages of coronary atherosclerosis (de Feyter et al. 1991). Once the plaque enlarges to >40% of the total vessel cross-sectional area, the artery no longer enlarges, and the lumen narrows as the plaque grows (Glagov et al. 1987).

Furthermore, conventional visual evaluation of the severity and extent of coronary obstructions is known to be greatly associated with high inter- and intraobserver variability (Zir et al. 1976, Marcus et al. 1988, Goldberg et al. 1990). In general, visual assessment leads to overestimation of the degree of narrowing in severe lesions and to underestimation of the severity of mild to moderate lesions (Fleming et al. 1991).

Interpretation of the severity and extent of CAD is hampered by variation among methods of classification of CAD phenotype (Selzer 1982, Gensini 1983). The definition of CAD severity was earlier based on descriptive terms such as: “marked” “significant” “mild” “moderate” “severe” (Waller et al. 1980). The traditional one-, two-, and three-vessel classification has also been considered inadequate (Selzer 1982). In addition, various semi-quantitative scoring systems have been used to make reporting on CAD severity more consistent (Gensini 1983, Moise et al. 1988, Sullivan et al. 1990).

In general, analysis of two or more orthogonal projections has been recommended to allow a more accurate assessment of the severity of lesions. However, adequate orthogonal views are frequently unobtainable due to vessel foreshortening, overlapping side branches, poor image quality, or disease at bifurcation sites (Topol et al. 1995). Videodensitometry is theoretically less vulnerable to inaccuracies. With this method, the contrast density of a selected reference segment is compared with the contrast density in the region of a stenosis. The major advantage of videodensitometry is that lesion eccentricity and irregularity can be accounted for without the need for multiple image projections. It requires, however, homogeneous complete opacification of the lumen and the clinical value of videodensitometry remains controversial (Alfonso 2000).

2.4.2. Computer-based quantitative coronary angiography

Computer-assisted quantitative analysis of coronary angiograms was developed to overcome the limitations of visual interpretation. Originally described in the late 1970s (Brown et al. 1977), QCA has rapidly displaced visual analysis in interventional studies and also in the estimation of the progression or regression of CAD (Reiber et al. 1993).

In terms of accuracy and reproducibility, QCA is superior to visual analysis of coronary angiograms. Variability in repeated measures of PDS or in absolute coronary dimensions using several different computer algorithms has shown minimal inter- or intraobserver variability (Brown et al. 1977, Cashin et al. 1984, Reiber et al. 1993). In a comparison between a panel with three readers and QCA, the latter method identified and measured diameter stenoses between 21% and 40%, which were not identified by the panel (Mack et al. 1992). A change of 20% in PDS has been shown to represent true progression or regression of coronary atherosclerosis with more than 95% confidence in QCA (Syvänne et al. 1994b).

Online computer-based QCA is possible with modern digital angiography equipment, and provides a tool in diagnostic and/or therapeutic decision making during the catheterization procedure. Offline methods for QCA are useful especially for scientific purposes. When cineangiograms recorded on film are used in computer-based QCA analysis, they first need to be transformed into digital information. Briefly, the subsequent steps of this analysis are: calibration of the image data, definition of coronary segment to be analyzed, and automated detection of the arterial contours, with or without user-defined corrections (Reiber et al. 1993).

Calibration needs to be carried out prior to analysis to allow measurement of absolute vessel dimensions. This procedure, that yields a calibration factor, is based on the known French catheter size and on measurement of the contrast-filled catheter with use of automated edge detection of this catheter. The first step in the actual coronary measurement is to define the start- and end-point of the segment under analysis. After that, the arterial contours are detected by the software, and thereafter several clinically relevant parameters are automatically calculated (Figure 5).

Neither visual nor computer-assisted techniques can correct for the inherent limitations of a silhouette technique. Coronary intravascular ultrasound (IVUS), developed over the past decade, has many potential advantages in characterizing the atherosclerotic disease process. IVUS is a catheter-based technique, which provides real-time high-resolution images allowing precise tomographic assessment of lumen area, plaque size, and composition of a coronary segment (Topol et al. 1995).

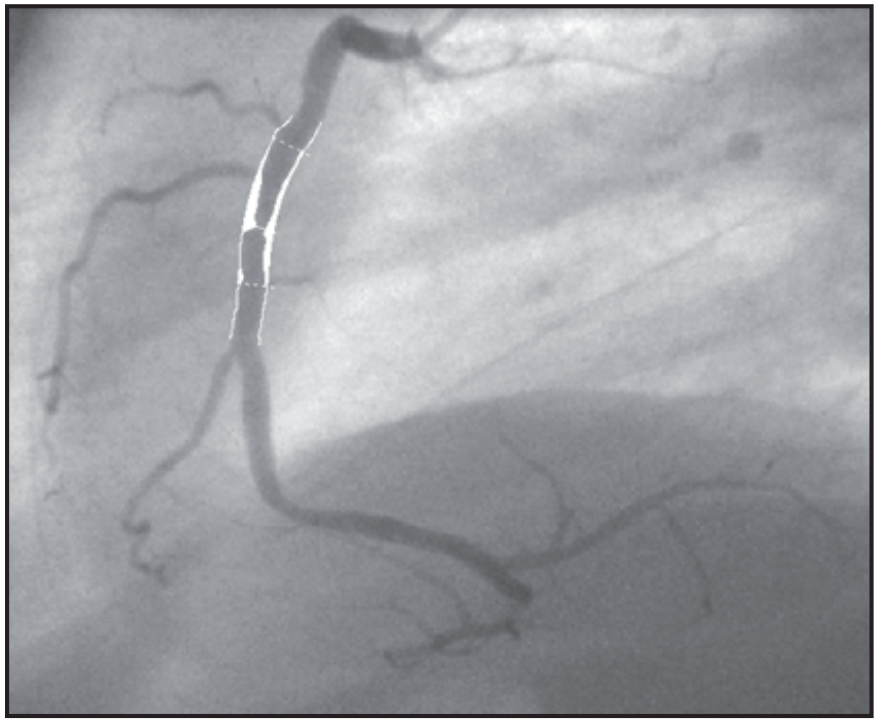
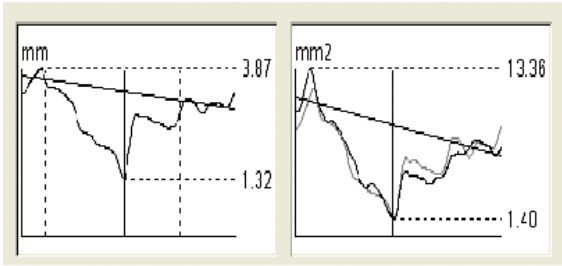


Figure 5. A final results page of QCA-CMS® analysis of the mid-part of a right coronary artery. In the upper panel and the lower left panel are the automatically detected luminal contours, the computer-defined reference contours, and the diameter function. In the right lower panel, the results page of the QCA analysis displayed with all detailed quantitative data. The percent diameter stenosis here was 61%.



MLD	:	**1.32**	mm
%D-Sten.	:	**61**	%
Ref. Diam	:	**3.34**	mm
Obstr. Length	:	**17.32**	mm
Pos. Prox. Border	:	3.72	mm
MLA Dens.	:	1.40	mm2
MLA Geom.	:	1.36	mm2
%A-Sten. Dens.	:	**84**	%
%A-Sten. Geom.	:	**84**	%
Ref. Area	:	8.78	mm2
Obstr. Volume	:	86.48	mm3
Plaque Area	:	14.19	mm2
Plaque Volume	:	63.61	mm3
Symmetry	:	0.64	

2.4.3. Ultrasonographic measurement of carotid artery intima-media thickness (IMT)

2.4.3.1. Overview

Over the past decade, the measurement of carotid IMT using high resolution B-mode ultrasonography has emerged as a method of choice for determining the anatomic extent of atherosclerosis and for assessing cardiovascular risk. B-mode ultrasonography is a relatively simple, inexpensive, and non-invasive method for determining atherosclerosis. Unlike angiography, ultrasound allows imaging of all stages of atherosclerosis including also early arterial vessel wall changes.

2.4.3.2. Measurement of carotid IMT

Ultrasound imaging cannot discriminate between the intima and the media of the vessel wall because of insufficient axial resolution (Simon et al. 2002). Therefore, the combined thickness of the intima and media, the intima-media complex, is widely applied. Notably, this approach does not entirely exclude the possibility of fibromuscular hypertrophy of the arterial media behind an increased carotid IMT (Glagov et al. 1988). In fact, hypertension has been shown to increase IMT independent of typical atherosclerotic changes, probably because of medial hypertrophy (Roman et al. 1995).

Current ultrasound instrumentation with transducers ≥8 MHz are capable of identifying the two-parallell echogenic lines (double line pattern), which correspond to the interfaces between vessel lumen and the intima as well as between the media and the adventitia. The thickness of the echogenic line next to the vascular lumen added to the thickness of the adjacent dark layer compose the IMT both at the near wall (NW) and at the far wall (FW). The screening examination is performed bilaterally on the extracranial carotid artery segments. These segments are the distal straight 1 cm of the common carotid arteries, the carotid bifurcations, and the proximal 1 cm of the internal carotid arteries (Greenland et al. 2001) (Figure 6 A-B).

The validity of the FW IMT measurements has been shown by comparing the IMT obtained by ultrasound imaging with the IMT determined by microscopy in pathologic evaluation (Pignoli et al. 1986, Wendelhag et al. 1991, Wong et al. 1993). In the NW measurement, IMT is 80% of the histological thickness, and a difference of 0.02 mm is present when comparing the NW and FW measurements (Kanters et al. 1997). Thus, the validity of NW measurements can be debated for several reasons.

An echo is produced by tissue interfaces with a sufficient difference in acoustic impedance. The anatomical location of a structure is determined by the leading edge (the upper edge) of the echo. Thus, thickness of an anatomical structure is defined as the distance between the leading edges of two different echoes. Normally, the adventitia is quite echogenic in contrast to the media. In the FW the interface between lumen and intima and the media-adventitia, respectively, are visualized. In the NW, the intima-lumen interface is usually well defined. The bright echoes produced by the adventitia,

however, overlap the echo originating from the adventitia-media interface, which cannot therefore be accurately visualized (Wendelhag et al. 1991). Moreover, the precision of the NW IMT depends on the axial resolution of the equipment used and on the gain setting (the higher the axial resolution, the more precise the measurement, the higher the gain, the lower the axial resolution) (Wendelhag et al. 1991). Although there is a small systematic difference between the NW and FW measurements, the annual IMT progression rates do not differ between the NW and the FW. In addition, including NW measurements reduces the variability of progression. Thus, combined measurements might enhance precision without loss of validity (Kanters et al. 1997).

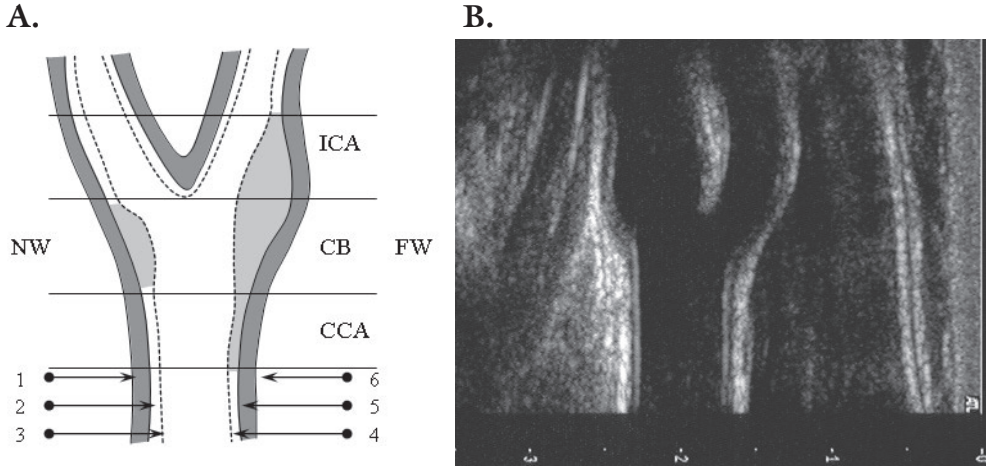


Figure 6 A-B. Schematic (A) illustration of the echoes seen in B-mode ultrasound images (B) of carotid artery. See text (chapter 2.4.3.) for explanation. NW; near wall, FW; far wall, ICA; internal carotid artery, CB; carotid bulb, CCA; common carotid artery.
Numbers indicate the acoustic interfaces as follows: 1. periadventitia-adventitia, 2. adventitia-media, 3. intima-lumen, 4. lumen-intima, 5. media-adventitia, 6. adventitia-periadventitia. The distance between points 2 and 3 corresponds to the NW- and between points 4 and 5 to FW IMT.
Modified and used with permission from Kati Ylitalo, Doctoral Thesis 2001.

Two main approaches are used for measuring IMT. Firstly, measurement can be restricted to the FW of the distal segment of the common carotid artery. This superficial and straight segment offers the best geometric conditions for obtaining high precision and reproducibility rate of ultrasound IMT measurement. IMT is not measured at a single point but averaged on approximately 100 points of measure along at least 1 cm of longitudinal length of the vessel. Notably, in the carotid artery, atherosclerotic lesions occur later in the common carotid artery than in the internal carotid artery or in the bifurcation (Solberg et al. 1971). Therefore IMT, when measured in the distal common carotid artery free from intrusive atherosclerotic plaque, may not be the most appropriate segment to study if the objective is focused on atherosclerosis (Simon et al. 2002). Secondly, IMT can be measured in the near

and far walls of the three main segments of extracranial carotid arteries (common carotid artery, carotid bifurcation, and internal carotid artery) on both sides. For each segment, ultrasound scan is performed in more than one direction, the maximal value of IMT is selected, and the final IMT considered is the average of IMT values at the multiple sites examined. Measurement of IMT at multiple carotid sites frequently incorporates plaque thickness because plaques are common in the carotid bifurcation and internal carotid artery. This explains why the measured IMT can be regarded as a marker of early carotid atherosclerosis (Simon et al. 2002).

2.4.3.3. Normal values of carotid IMT

In general, greater IMT values are associated with greater cardiovascular risk. For example, a recent meta-analysis found that the future risk of myocardial infarction increases by 10% to 15% and the stroke risk by 13% to 18% for each 0.1 mm increase in carotid IMT (Lorenz et al. 2007). Despite this continuous relationship between IMT and risk, there is no clear cut-off point for the definition of an abnormally high IMT. IMT increases with age and is generally thicker in men than in women (Cheng et al. 2002). Accordingly, designation of what is abnormal must consider at least these basic issues. The normal values of carotid IMT have generally been established on the basis of the distribution of IMT values (histogram) within a general healthy population. Hence, the upper normal limit used for defining normal range of IMT is arbitrary and is frequently set at the age-adjusted 75th upper percentile of the IMT distribution. The epidemiological data currently available indicate that a value of carotid IMT at or above 1 mm at any age is associated with a significantly increased risk of myocardial infarction and/or cerebrovascular disease (Simon et al. 2002).

2.4.3.4. Carotid IMT and cardiovascular risk factors

In observational and epidemiological studies conducted in the general population traditional risk factors, such as ageing, male sex, hypertension, elevated total and LDL cholesterol levels, low HDL cholesterol level, DM, and smoking are associated with increased carotid IMT (Salonen et al. 1991, Crouse et al. 1996, Gariepy et al. 1998, Lakka et al. 1999, Espeland et al. 1999).

Numerous studies have investigated the association between carotid IMT and new or emerging risk factors. In general, data on the relationship between PON1 Q/R192 polymorphism and carotid IMT have been negative in healthy individuals (Schmidt et al. 1998, Dessi et al. 1999, Markus et al. 2001) or in type 2 diabetic subjects (Cao et al. 1998). Likewise, the results on the connection between carotid IMT and PON1 activity and concentration have been conflicting (Jarvik et al. 2000, Valabhji et al. 2001, Campo et al. 2004).

A link between IR and carotid IMT has been addressed in several studies, but the results are contradictory (Suzuki et al. 1996, Shinozaki et al. 1997, Ishizaka et al. 2003). Studies on the relation between carotid IMT and Lp(a) have yielded conflicting results (Schreiner et al. 1996, Grebe et al. 2006). A positive relationship be-

tween carotid IMT and ε4 allele has been shown in some studies (Terry et al. 1996, Cattin et al. 1997, Elosua et al. 2004), although others have failed to find such a relationship (Slooter et al. 2001, Fernandez-Miranda et al. 2004).

Moreover, a significant association between LDL particle size and carotid IMT has been shown in middle-aged healthy subjects (Skoglund-Andersson et al. 1999, Hulthe et al. 2000a), but not in subjects with hypercholesterolaemia (Hulthe et al. 2000b) or with CAD risk among elderly subjects (Mykkänen et al. 1999). Similarly, the data on associations between carotid IMT and circulating oxLDL or autoantibodies against oxLDL have been inconsistent (Salonen et al. 1992, Uusitupa et al. 1996, Hulthe et al. 2002, Wallenfeldt et al. 2004, Mayr et al. 2006). Increased carotid IMT has been associated with postprandial lipidemia (Karpe et al. 1998, Boquist et al. 1999, Karpe et al. 2001).

2.4.3.5. Carotid IMT and cardiovascular disease

Several large-scale prospective studies in general population have examined the relationship between carotid IMT and the incidence of cardiac and cerebrovascular events. A recent meta-analysis based on data from 37 197 subjects, who were followed up for a mean of 5.5 years, fully supports the impact of increased carotid IMT as an independent predictor of future vascular events (Lorenz et al. 2007).

Carotid IMT has been employed to assess the potential therapeutic impact of statins on the progression of carotid atherosclerosis. Indeed, a recent meta-analysis, including seven placebo-controlled clinical trials of statins, reports that statin therapy was associated with an average decrease of IMT progression of 0.012 mm/year with 95% confidence interval [-0.016, -0.007]. More importantly, the meta-analysis yields a significant odds ratio of 0.48 [0.30, 0.78] for the reduction of cardiovascular events associated with statin therapy (Espeland et al. 2005).

The positive association between carotid IMT and angiograhic measures of CAD has been addressed in numerous studies, but the results have been contradictory (Wofford et al. 1991, Tanaka et al. 1992, Adams et al. 1995, Lekakis et al. 2000, Holaj et al. 2003). Wofford et al. (1991) found in 843 patients undergoing coronary angiography for clinical purposes a strong relation between extent of carotid atherosclerosis, as measured by B-mode ultrasound, and extent of coronary atherosclerosis, as measured by visual interpretation of coronary angiograms. Adams et al. (1995) concluded, in 350 subjects referred for coronary angiography, that carotid IMT and angiographically assessed extent and severity of CAD is only weakly correlated.

Only a few studies (Blankenhorn et al. 1993, Herrington et al. 1994, Mack et al. 2000) have examined the correlation between carotid IMT and CAD utlilizing quantitative imaging. Again, results from these studies have been conflicting. Herrington et al. (1994), who examined 86 patients with the B-mode score (mean of the maximum IMT at 12 sites) and QCA (percent diameter stenosis), found a correlation coefficient of r=0.27. Moreover, Mack et al. (2000), who investigated 188 non-smoking males with prior coronary artery bypass grafting, found no significant correlations between common carotid artery IMT and QCA measures.

3. AIMS OF THE STUDY

To assess quantitatively the severity and extent of coronary and carotid artery atherosclerosis and to correlate these measures to potential risk factors in a Finnish patient sample referred for clinically indicated coronary angiography.

The specific aims of the study were to:

1) Examine the relation between carotid IMT and angiographic severity and extent of CAD.
2) Define the correlation between measures of coronary and carotid atherosclerosis and novel lipoprotein-related parameters such as PON1.
3) Investigate the association between measures of coronary and carotid atherosclerosis and remnants of TRLs in the postprandial state.
4) Discover the connection between measures of coronary and carotid atherosclerosis and LDL oxidation parameters.
5) Elucidate the relation between measures of coronary and carotid atherosclerosis and measures of IR.
6) Scrutinize the link between measures of coronary and carotid atherosclerosis and apoE polymorphism.

4. SUBJECTS AND STUDY DESIGN

This cross-sectional study comprised 108 patients referred for elective coronary angiography at Helsinki University Central Hospital between March 1999 and December 2003. The study design included coronary angiography, ultrasound imaging of carotid arteries, extensive fasting blood samples, oral glucose tolerance test, and an oral fat-load test to be performed in each participant.

To be eligible for the study, the participants had to be male or female 35 to 75 years old with clinically suspected CAD (previous myocardial infarction and/or positive exercise test). The aims of these criteria were to include a representative, fairly unselected study sample and a wide range of atherosclerosis severity. Twenty-four participants had type 2 DM, of whom 17 were treated with diet alone, two with metformin, three with sulfonylureas, and two with a combination of metformin and sulfonylureas. The mean duration of type 2 DM was 2.4 ±2.1 years.

The following exclusion criteria were applied: 1) patients referred primarily for evaluation of valvular or other structural heart disease, for arrhythmia evaluation, or consideration of heart transplantation, 2) previous coronary artery bypass grafting or percutaneous coronary intervention, 3) type 1 DM, 4) significant renal failure (serum creatinine >150 μmol/L), and 5) physical, psychological, or logistic problems to participate in all protocol-defined procedures. One patient was excluded in studies II-V due to severe hypertriglyceridemia (TGs >14 mmol/L).

Written informed consent was obtained from all participants and the study design was approved by the institutional ethics committee.

5. METHODS

5.1. Demographic variables

Study participants completed standard questionnaires to provide data on previous medical history, medication, smoking habits, and symptoms of CAD. Functional class was determined according to the classification of the Canadian Cardiovascular Society (Campeau 1976). Presence of hypertension was defined as current use of antihypertensive drugs. Smoking status was recorded as those who smoked one or more cigarettes a day and those who had quit smoking or never smoked (studies I, III). The patients were also classified as either nonsmokers or as past or present smokers (studies II, IV, V). Body mass index was calculated by dividing weight in kilograms by height in meters squared (kg/m^2). The waist circumference was used as a measure of intra-abdominal fat deposition.

5.2. Biochemical analyses

Blood samples were collected one month after coronary angiography after an overnight fast. To avoid bias caused by medications, the patients were requested to abstain from any lipid-lowering drugs between the time of angiography and blood sampling; only highly cardioselective β blockers (mainly bisoprolol) were permitted.

5.2.1. Lipid and lipoprotein measurements

Serum and ethylenediaminetetraacetic acid plasma were separated by centrifugation and stored at – 80°C until analyzed. Cholesterol and TG levels were measured by automated enzymatic procedures (Hoffman-La Roche, Basel, Switzerland). The LDL, HDL, HDL_2, and HDL_3 cholesterol levels were determined after separating the lipoprotein fractions from fresh fasting sera by sequential ultracentrifugation (Taskinen et al. 1988). Concentrations of apoA-I, apoA-II, apoB, and Lp(a) were measured by immunoturbidimetric methods with commercial kits (Boehringer-Mannheim, Mannheim, Germany). LpA-I-particles were quantified using a differential electroimmunoassay (Sebia, Issy-les-Moulineaux, France) (Parra et al. 1990). The concentration of LpA-I/A-II-particles was calculated by subtracting the concentration of LpA-I from the total concentration of apoA-I in serum. LDL peak particle diameter (LDL particle size) was determined by a non-denaturing polyacrylamide gel electrophoresis (Vakkilainen et al. 2002a). A monoclonal antibody 4E6-based competition ELISA was used for measuring plasma levels oxLDL (Holvoet et al. 2001). RLP-C was measured by an immunoseparation method using anti-human apoB-100 and anti-human apoA-1 monoclonal antibodies (Japan Immunoresearch Laboratories, Takasaki, Japan).

ApoE phenotyping was performed in serum by using the method of Havekes et al. (1987). ApoE concentration was determined using ELISA. Microtiter wells were coated with rabbit anti-human apoE IgG (R 107, rabbits were immunized with apoE purified from human plasma). After sample incubation the bound apoE was detected with second antibody, horseradish peroxidase-conjugated rabbit anti-human apoE (DAKO A0077) (Siggins et al. 2003).

PON-1 activity, using phenylacetate as substrate, was analyzed in serum samples as described previously (Blatter Garin et al. 1994, Blatter Garin et al. 1997). The serum concentration of PON1 was assayed using a competitive ELISA (Blatter Garin et al. 1994).

5.2.2. Glucose and insulin measurements

All subjects underwent a 75-g standard oral glucose tolerance test, and blood specimen were collected before and 60, and 120 minutes after loading for determination of plasma glucose and serum insulin concentrations. Fasting and post-load glucose were measured by the hexokinase method (Roche Diagnostic Gluco-quant) using either a Hitachi 917 or a Modular analyser (Hitachi Ltd, Tokyo, Japan). Serum insulin concentrations were determined by double-antibody radioimmunoassay (Pharmacia RIA kit, Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol. Abnormal glucose regulation was defined as a history of known DM or as a fasting plasma glucose ≥6.1 mmol/L or as 2-hour plasma glucose ≥7.8 mmol/L according to World Health Organization (WHO) criteria (1999).

To assess IR in the non-diabetic subjects, HOMA was calculated by using the following formula: HOMA IR = fasting insulin (mU/L) x fasting glucose (mmol/L)/ 22.5 (Matthews et al. 1985).

5.2.3. Oral fat-load test and separation of TRL fractions

The study subjects consumed a high-fat meal comprising bread, butter, cheese, sliced sausage, boiled egg, paprika, soured whole milk, orange juice, and coffee. The energy content of this test meal was 65% fat, 20% carbohydrate, and 15% protein, and it was ingested within 10 minutes. The cholesterol intake was 490 mg. The fatty acid composition of the fat consumed was 65% saturated fat, 30% monounsaturated fat, and 5% polyunsaturated fat leading to a ratio of polyunsaturated fat to saturated fat of 0.08. Baseline blood samples were drawn before the meal after an overnight fast. Postprandial blood samples were obtained at 6 hours after the fatty meal.

TRLs were isolated by density-gradient ultracentrifugation (Syvänne et al. 1993). The following lipoprotein fractions were separated: the Sf>400 fraction representing CMs and large VLDLs, and the Sf 12-400 fraction corresponding to the typical VLDL and IDL density in fasting plasma. Concentrations of apoB-48 and apoB-100 were analyzed from TRL fractions (Mero et al. 2000).

5.3. Ultrasonographic measurement of carotid IMT

B-mode ultrasound imaging was performed with a Hewlett-Packard Image point M2410A ultrasound system (Hewlett-Packard, Andover, USA) equipped with a 10 MHz linear array transducer and videotaped with a Panasonic AG-MD830E PAL S-VHS VCR. All examinations were carried out by the same operator, who was blinded to the quantitative coronary angiographic results. Subjects were examined in the supine position. Longitudinal images from three angles of interrogation (anterolateral, lateral, and posterolateral) were displayed bilaterally for the common carotid artery, carotid bifurcation, and internal carotid artery. Measurements were carried out at a total of 28 sites per patient from three projections for both the FW and the NW of the common carotid artery and carotid bifurcation, and the one best visualized projection for internal carotid artery. The images were frozen in the diastole, assessed as the phase when lumen diameter is at its smallest and IMT at its largest.

Computer analysis of ultrasound images was performed by a single reader at the Research Institute of Public Health, Kuopio, Finland. The reader´s repeatability has been assessed earlier in another study with an identical carotid ultrasound protocol (Ylitalo et al. 2002). IMT measurements from videotapes were made at a total of 28 sites corresponding to the 28 sites where the scanning was focused. The mean, maximum, and minimum IMT were derived from each measurement. For each subject the mean IMT was calculated as the average of all mean IMT measurements over 28 sites. Likewise, maximum IMT was calculated as the average of the thickest points recorded in each segment.

5.4. Coronary angiography

Coronary angiography was performed by the percutaneous femoral approach using standard angiographic techniques. The left and right coronary arteries were imaged in multiple projections to permit an adequate diagnostic study as well as a quantitative analysis. Sublingual nitroglycerin was routinely administered to control vasomotor tone.

5.4.1. Visual analysis of coronary angiograms

Angiographic scoring was performed by interventional cardiologists who were blinded to the study protocol. Mild CAD on visual interpretation was defined as lumen diameter reduction <50%, and significant CAD as the presence of any luminal stenosis ≥50%. Due to the fact that most therapeutic decisions are based on visual angiographic results in clinical practice, patients in publications I and II were divided into two subgroups such that those without any or with mild CAD comprised one group and those with ≥1 significantly affected coronary artery the other.

5.4.2. Frame selection for quantitative coronary angiography analysis

Coronary segments were analyzed in one angiographic view. The frames for QCA analysis were selected by one of the investigators using the following criteria: minimal foreshortening and overlap, good visualization of any stenoses within the segment, and optimal contrast and image quality available. Usually the selected frames were in end-diastole or in the diastasis period.

5.4.3. Segmental classification of the coronary tree

Classification of the coronary tree is illustrated in Figure 7. The coronary segments were classified into four categories based on their location. The left main coronary artery was analyzed separately. Proximal parts of the anterior descending, the left circumflex, and the right coronary arteries were considered proximal segments. Mid segments comprised the mid parts of the three main coronary arteries. All segments distal to the mid segments were regarded as distal segments. The distal segments included also the first and the second diagonal and obtuse marginal branches whenever these arteries were considered suitable for analysis by QCA. In addition, if large and well visible, we included the posterolateral branches of the left circumflex artery and the right coronary artery, the right ventricular branch and the acute marginal branch among the distal segments. Segments with a diameter of approximately ≤1.5 mm were excluded from analysis.

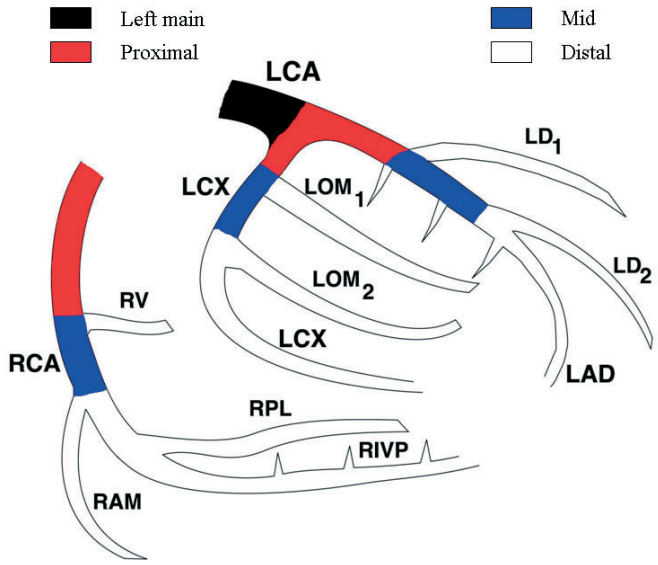


Figure 7. Segmental classification of the coronary tree. LCA, left main coronary artery; LAD, left anterior descending coronary artery; LD1, LD2, left diagonal branches; LCX, left circumflex coronary artery; LOM1, LOM2, left obtuse marginal branches; RCA, right coronary artery; RV, right ventricular branch; RAM, right acute marginal branch; RPL, right posterolateral branch; RIVP, right interventricular posterior branch.

5.4.4. Qantitative analysis of coronary angiograms

The coronary angiograms were analyzed using third-generation QCA software, the Cardiovascular Measurement System (QCA-CMS) version 3.0 (Medis, Nuenen, the Netherlands). This system has been described elsewhere in detail (Reiber et al. 1993) and has also been validated in our laboratory (Syvänne et al. 1994b, Pajunen et al. 1997). All QCA analyses were carried out by one of the investigators. The contrast-filled catheter lumen diameter was used as a calibration standard. A stenosis was considered to be present when the first analysis indicated a diameter narrowing of at least 20% (Syvänne et al. 1994b). An example of a quantitative analysis result page is presented in Figure 5.

5.4.5. Measures of severity, extent, and atheroma burden of CAD

Based on computer-aided analysis, we assessed the severity, extent, and overall atheroma burden indexes to describe per-patient characteristics of CAD (Figure 8). These three indexes were calculated for the entire coronary tree of each patient (global) and separately for the proximal, mid, and distal segments.

Severity: Severity refers to the tightest diameter stenosis within a certain coronary territory. It was defined as the narrowest lesion expressed in percentage diameter stenosis (ranging from zero if no stenosis was present to 100% in case of a total occlusion) in the left main, left anterior descending, left circumflex, and right coronary artery territory. The global PDS index was counted as the average of the most severe stenoses in these four vessels. The severity index was likewise calculated for proximal-, mid-, and distal segments of the coronary tree.

Extent: Extent was defined as the percentage of a coronary segment involved in a stenosis and was calculated as: 100 x stenosis length/segment length. If a segment contained >1 stenosis, the sum of the stenosis lengths was used. The extent indexes (global, left main, proximal, mid, distal) were calculated as sums of the pertinent stenosis length values divided by total length of segments available for analysis.

Atheroma burden: Atheroma burden was derived from the plaque area (expressed in square millimeters), which represents a two-dimensional projection of the area covered by atherosclerotic tissue within the stenosis. The sum of the QCA-derived plaque areas within a certain segment were divided by the respective segment lengths, and the global atheroma burden index was the sum of all the plaque areas measured divided by total length of all the segments available for analysis.

Total occlusions: To avoid excluding data on the most severely diseased vessels, extent and plaque areas of totally occluded segments were imputed as follows. For any occluded segment (e.g. proximal left anterior descending artery), the maximum stenosis length or plaque area measured in the corresponding segment of any patient was taken to represent the pertinent value for that segment. The denominator needed to calculate the extent and atheroma burden values was the length of the most severely diseased corresponding segment; however this was adjusted for the average lengths of those segments within the study group.

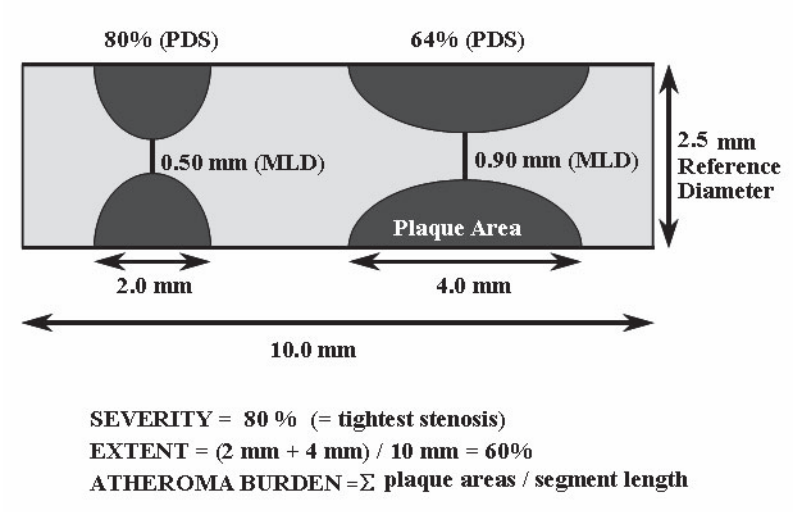


Figure 8. Schematic diagram of a coronary segment illustrating definitions of the angiographic indexes. The vessel (reference) diameter is 2.5 mm. The lesion on the left has a minimum luminal diameter (MLD) of 0.50 mm, and therefore a percent diameter stenosis (PDS) (measure of severity) of 80 %. The length of the lesion is 2.0 mm. The lesion on the right is less severe but longer. Segment length is 10 mm; thus extent in this segment is 100 x (2 + 4 mm)/10 mm = 60%. "Plaque areas" defining "atheroma burden" are shown in black.
Adapted with permission from Pia Pajunen, Doctoral Thesis 2002.

5.6. Statistical analyses

All statistical analyses were performed with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as frequencies or percentages for categorical variables and as mean ± standard deviation for continuous variables, unless otherwise noted. Normality of continuous variables was checked by the Kolmogorov-Smirnov test. Logarithmic tranformation of variable was done, if necessary. Correlations were calculated by the univariate Spearman correlation coefficients. Between-group differences were assessed by the Mann-Whitney U-test, the Kruskal-Wallis test, one-way analysis of variance, and the chi-square test, as appropriate.

Friedmans test (study I) was used to compare the severity and extent of CAD between proximal, mid, and distal vessel segments. Wilcoxons signed rank test was used (study III) to compare postprandial with fasting values. The means of continuous variables between different study groups (studies IV and V) were compared by using the analysis of covariance with adjustment for age and gender.

Multivariate linear regression analyses were employed to assess the predictors of global PDS index (studies I, II, and IV) or global atheroma burden index (studies III and V). To adjust for confounding, age and sex were included in all multivariate regression models. P values <0.05 were considered significant.

6. RESULTS

6.1. Association between coronary and carotid atherosclerosis (Study I)

6.1.1. Study population

The demographic and clinical characteristics of the study population are presented in Table 3. Typical atherosclerotic risk factors and symptoms of CAD were prevalent. Most patients had evidence of ischemia during exercise testing, and 39 (36%) patients had suffered a previous myocardial infarction. A high percentage of participants was taking aspirin, β blockers, and lipid-lowering drugs at baseline.

Table 3. Demographic and clinical characteristics of the study population (n=108).

Variable	Median (interquartile ranges) or frequencies (%)
Age (years)	61 (55-65)
Male/Female	81/27 (75%/25%)
Body mass index (kg/m^2)	27.2 (25.0-29.0)
Total cholesterol (mmol/L)	5.57 (4.72-6.50)
HDL cholesterol (mmol/L)	1.31 (1.13-1.50)
LDL cholesterol (mmol/L)	3.37 (2.75-4.17)
Triglycerides (mmol/L)	1.77 (1.32-2.42)
Fasting plasma glucose (mmol/L)	5.5 (5.1-6.3)
2-hour plasma glucose (mmol/L)	6.8 (5.4-8.9)
Current smoker	18 (17%)
Hypertension	63 (58%)
Abnormal glucose regulation	44 (41%)
Previous myocardial infarction	39 (36%)
Previous cerebrovascular disease	4 (4%)
Positive exercise test	85 (79%)
Canadian Cardiovascular Society class	
I	7 (6%)
II	46 (43%)
III	50 (46%)
IV	5 (5%)
Medication use	
Aspirin	100 (93%)
β blocker	91 (84%)
Statin	77 (71%)
Angiotensin-converting enzyme inhibitor	31 (29%)
Calcium channel blocker	21 (19%)
Diuretics	14 (13%)
Long-acting nitrates	50 (46%)
Later treatment of CAD	
Medical	37 (34%)
Percutaneous coronary intervention	44 (41%)
CABG	27 (25%)

HDL, high-density lipoprotein; LDL, low-density lipoprotein; CAD, coronary artery disease; CABG, coronary artery bypass grafting.

6.1.2. Visual angiographic and quantitative coronary angiographic results

On visual interpretation only 11 (10%) subjects had normal coronary arteries. Mild CAD was diagnosed in 12 participants (11%), one-vessel CAD in 28 (26%), two-vessel CAD in 27 (25%), and three-vessel CAD in 30 (28%). Fourteen subjects had significant CAD in the left main coronary artery.

The global PDS index was 30.9±13.6 (range 0 to 60.4), the global extent index was 14.0±6.8 (range 0 to 31.3), and the global atheroma burden index was 7.5±4.8 (range 0 to 23.2). PDS indexes were 14.6±13.2 (range 0 to 62.3) for proximal segments, 20.7±16.9 (range 0 to 77.4) for mid segments, and 19.3±12.1 (range 0 to 53.6) for distal segments. Extent indexes were 15.2±12.9 (range 0 to 46.1) for proximal segments, 17.0±13.0 (range 0 to 46.7) for mid segments, and 13.0±7.5 (range 0 to 42.6) for distal segments. Atheroma burden indexes were 10.2±11.1 (range 0 to 54.9) for proximal segments, 10.8±11.1 (range 0 to 45.3) for mid segments, and 5.9±4.4 (range 0 to 20.6) for distal segments. PDS index was lower in the proximal segment than in the mid and distal segments (p=0.003). We found, however, no significant differences between the types of vessel segment if the extent or the atheroma burden index was used (p=0.269 and p=0.116, respectively).

6.1.3. Relation between carotid IMT and severity and extent of CAD

In univariate analyses, maximum IMT values were significantly associated with the QCA-derived global indexes for the severity, extent, and atheroma burden of CAD (Figure 9 A-C). We found higher maximum IMT values (1.35±0.23 versus 1.20±0.20 mm, p=0.013) in the group with significant CAD as compared with those with no or mild CAD. The result was similar when mean instead of maximum IMT value was used (1.07±0.19 versus 0.95±0.16 mm, p=0.013). Further, means of maximum IMT were 1.20±0.20 mm in mild CAD, 1.27±0.22 mm in one-vessel CAD, 1.40±0.22 mm in two-vessel CAD, and 1.40±0.24 mm in three-vessel CAD (p=0.005). The outcome was essentially comparable when mean of mean instead of mean of maximum IMT was used.

Interestingly, we found heterogeneity in associations between IMT and CAD indexes according to anatomical location of CAD. Maximum and mean IMT values, respectively, were correlated with quantitative angiographic indexes for mid and distal segments only and not for left main coronary artery or proximal segments.

Analysis based on quartiles of the distributions of maximum and mean IMTs, respectively, suggested a non-linear relationship with global CAD severity so that only the lowest IMT values were related to mild CAD (Figure 10 A-B). Global extent and atheroma burden indexes showed similar associations (data not shown).

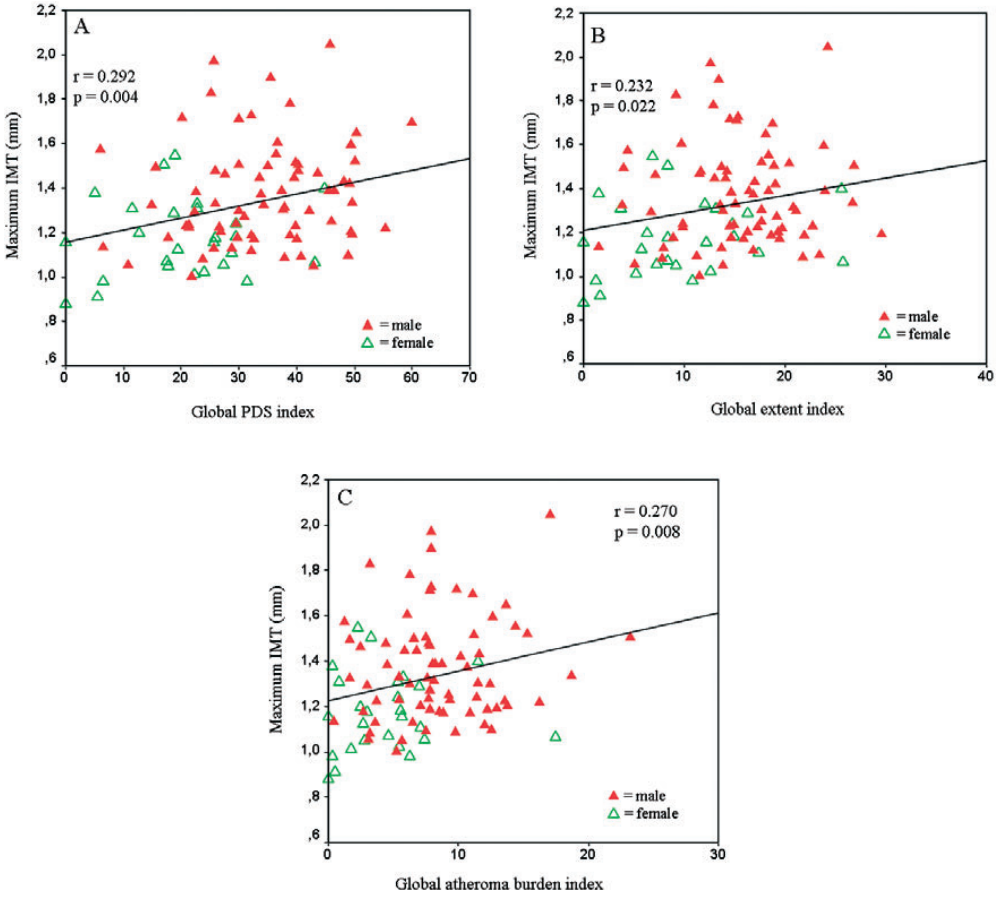


Figure 9 A-C. Correlations among maximum IMT (n=97) and global percent diameter stenosis (PDS) index (A), global extent index (B), and global atheroma burden index (C). For definition of the indexes, see text (chapter 5.4.5.).
Reprinted from the original publication (I) with the permission from the publisher (Elsevier).

In multivariate analysis only smoking ($p<0.001$), and to a lesser degree, age ($p=0.084$) were significant predictors of the global PDS index, whereas hypertension, total cholesterol, and abnormal glucose regulation were not. The model accounted for 14.2% of the variation of the global PDS index. When the variable maximum IMT was added, the accountability of the model increased by 5.9 percentage points. Not surprisingly, the addition of gender, further increased the predictive power of the model, but maximum IMT no longer retained its independent predictive power, presumably because of an overriding influence of sex in the model. In contrast, there was no evidence of interaction between maximum IMT and gender terms in a further analysis with global PDS index as the dependent variable, suggesting that the relation between IMT and CAD severity is similar in men and women.

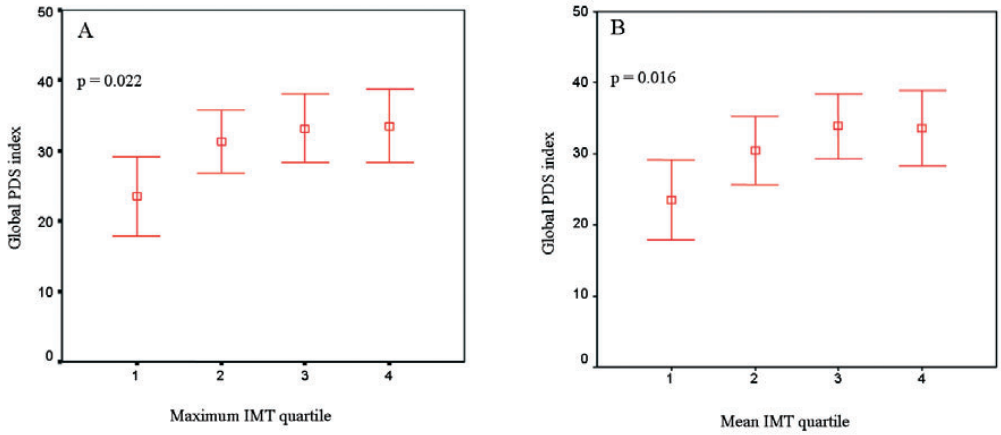


Figure 10 A-B. Relation between global percent diameter stenosis (PDS) index by maximum IMT quartile (A) and mean IMT quartile (B). Values of global PDS index are presented as mean ±SEM. For definition of the index, see text (chapter 5.4.5).
Reprinted from the original publication (I) with the permission from the publisher (Elsevier).

6.2. PON-1 activity and concentration and coronary and carotid atherosclerosis (Study II)

6.2.1. PON1 activity and concentration and clinical and lipid variables

In univariate correlation analyses PON1 activity and concentration were significantly correlated with HDL cholesterol ($p=0.005$ for both), apoA-I ($p<0.001$ and $p=0.006$, respectively), apoA-II ($p<0.001$ for both), and LpAI-/A-II ($p=0.001$ and $p=0.016$ respectively). The HDL_3 cholesterol level was associated with borderline significance to PON1 activity and concentration ($p=0.050$ and $p=0.065$, respectively). In addition, PON1 activity was associated with gender ($p=0.035$), abnormal glucose regulation ($p=0.045$), and LpA-I ($p=0.013$) (Table 4).

Table 4. Correlation coefficients between PON1 activity and concentration and selected variables.

	PON1 activity (U/mL)		PON1 concentration (ug/mL)	
	r	p	r	p
Age (years)	-0.150	0.123	-0.132	0.175
Gender	0.204	0.035	0.184	0.058
Hypertension	-0.100	0.307	-0.165	0.088
Current or former smoker	-0.112	0.250	-0.143	0.142
Abnormal glucose regulation	-0.194	0.045	-0.115	0.237
Total cholesterol	0.075	0.444	0.161	0.097
HDL cholesterol	0.268	0.005	0.270	0.005
HDL_2 cholesterol	0.150	0.124	0.170	0.080
HDL_3 cholesterol	0.190	0.050	0.179	0.065
LDL cholesterol	-0.034	0.728	0.044	0.653
VLDL cholesterol	0.049	0.618	0.056	0.565
Triglycerides	0.084	0.387	0.107	0.272
ApoA-I	0.355	<0.001	0.263	0.006
ApoA-II	0.368	<0.001	0.386	<0.001
ApoB	-0.034	0.725	0.080	0.412
LpA-I	0.240	0.013	0.178	0.067
LpA-I/A-II	0.304	0.001	0.232	0.016
Global PDS index	-0.364	<0.001	-0.306	0.001
Global extent index	-0.221	0.022	-0.161	0.097
Global atheroma burden index	-0.277	0.004	-0.229	0.017
Mean carotid IMT	0.018	0.860	-0.094	0.360
Maximum carotid IMT	0.008	0.939	-0.107	0.298

PON1, paraoxonase-1; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; ApoA, apolipoprotein A; ApoB, apolipoprotein B; LpA, lipoprotein A; PDS, percent diameter stenosis; IMT, intima-media thickness.

6.2.2. PON1 activity and concentration, severity and extent of CAD, and carotid IMT

As outlined in Table 4, PON1 activity was significantly and inversely associated with the QCA-derived global indexes for the severity ($p<0.001$), extent ($p=0.022$), and global atheroma burden ($p=0.004$). Similarly, PON1 concentration correlated significantly with global PDS index ($p=0.001$) and global atheroma burden index ($p=0.017$). Neither PON1 activity nor concentration was associated with carotid IMT.

We found lower values of PON1 activity (84±2 versus 100±5 U/mL, $p=0.003$) and PON1 concentration (94±3 versus 110±6 ug/mL, $p=0.016$), respectively, in the group with significant CAD as compared with the group with no or mild CAD.

Furthermore, mean values of QCA-derived global indexes for the severity, extent, and atheroma burden of CAD decreased in a stepwise manner across the tertiles of PON1 activity (Figure 11 A-C). Similar associations were found when tertiles of PON1 concentration instead of tertiles of PON1 activity were used (data not shown).

To establish independent determinants of global PDS index, we performed a linear regression analysis controlled for age, gender, hypertension, abnormal glucose regulation, smoking status, HDL cholesterol, and PON1 activity. In the final model that explained 25.1% of variation of global PDS index, the most important determinants were gender (p=0.001) and PON1 activity (p=0.016). The outcome was similar when replacing HDL cholesterol with HDL_2 cholesterol, HDL_3 cholesterol, apoA-I, or apoA-II (data not shown).

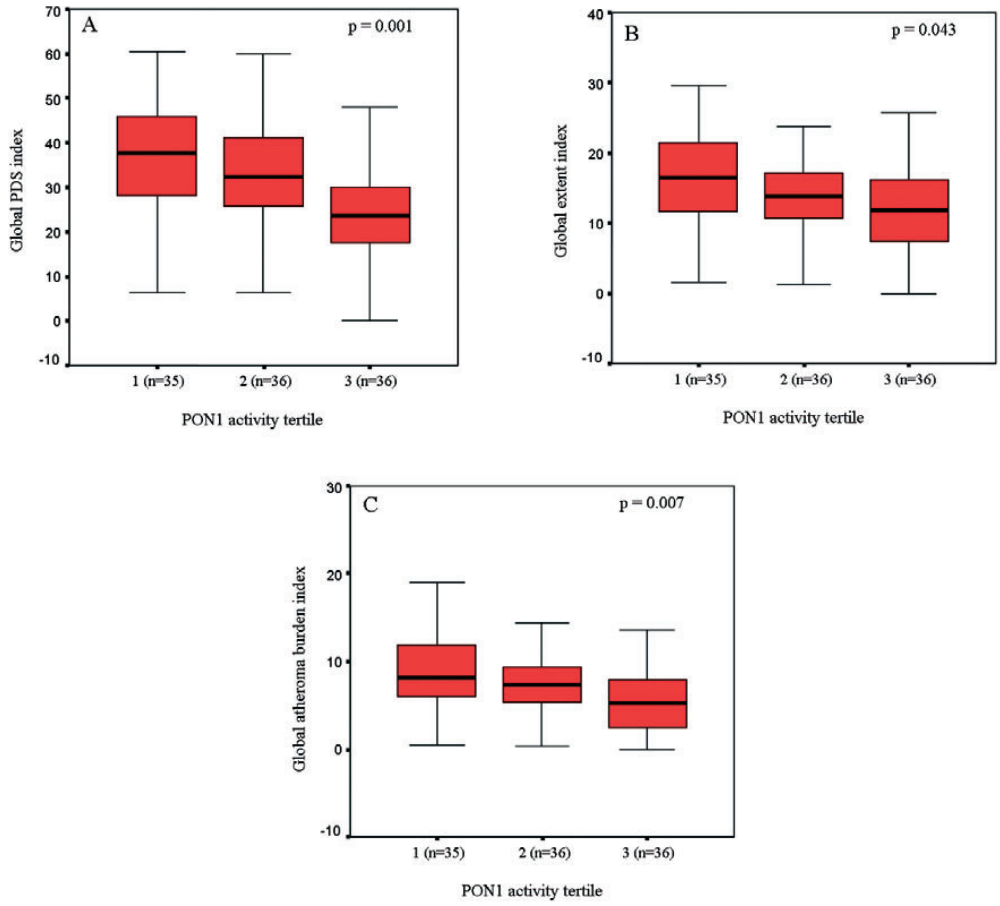


Figure 11 A-C. Relationship between paraoxonase-1 (PON1) activity tertile by global percent diameter stenosis (PDS) index (A), global extent index (B), and global atheroma burden index (C), respectively. For definition of the indexes, see text (chapter 5.4.5.). Reprinted from the original publication (II) with the permission from the publisher (Elsevier).

6.3. Postprandial lipemia (Study III)

6.3.1 Postprandial responses of plasma TGs and TRLs

The level of plasma TGs was significantly increased at 6 hours after intake of oral fat meal as compared to fasting value (2.96±0.13 versus 2.23±1.12 mmol/L, $p<0.001$). Similarly the concentration of TGs in CM + large VLDL (Sf>400) and in VLDL + IDL (Sf 12-400) fractions increased postprandially (0.35±0.03 versus 0.10±0.02 mmol/L and 1.70±0.09 versus 1.32±0.09 mmol/L, respectively, $p<0.001$ for both). The apoB-48 concentration also increased at 6 hours in CM + large VLDL (Sf>400) and especially in VLDL + IDL (Sf 12-400) fractions compared to fasting values (0.21±0.02 versus 0.05±0.01 mg/L and 14.95±1.11 versus 8.86±0.80 mg/L, respectively, $p<0.001$ for both). Likewise, the apoB-100 concentration rose postprandially in CM + large VLDL (Sf>400) and in VLDL + IDL (Sf 12-400) fractions (0.49±0.06 versus 0.33±0.05 mg/L, $p<0.001$ and 661±44 versus 624±42 mg/L, $p<0.05$, respectively).

6.3.2. Postprandial responses of RLP-C, oxLDL, and LDL particle size

The concentration of RLP-C was elevated 6 hours after oral fat meal as compared to baseline value (13.73±1.08 versus 10.16±0.90 mmol/L, $p<0.001$). OxLDL was also markedly increased 6 hours postprandially (104±2 versus 92±3 U/L, $p<0.001$). The mean peak particle size of LDL remained unchanged 6 hours after test meal (25.1±0.1 versus 25.1±0.1 nm, $p=0.099$).

6.3.3. Correlations between postprandial lipoproteins and other selected variables

A strong correlation existed between RLP-C and total TGs in fasting and in fed state (Figure 12 A-B). Likewise, the postprandial RLP-C correlated with respective TGs in Sf>400 ($r=0.737$, $p<0.001$) and Sf 12-400 ($r=0.857$, $p<0.001$), apoB-48 in Sf>400 ($r=0.710$, $p<0.001$) and Sf 12-400 ($r=0.664$, $p<0.001$), apoB-100 in Sf > 400 ($r=0.812$, $p<0.001$) and Sf 12-400 ($r=0.533$, $p<0.001$).

Interestingly, RLP-C correlated with oxLDL both in fasting and in fed state (Figure 13 A-B). There was an inverse relationship between RLP-C and LDL particle size before and 6 hours after oral fat load (Figure 14 A-B).

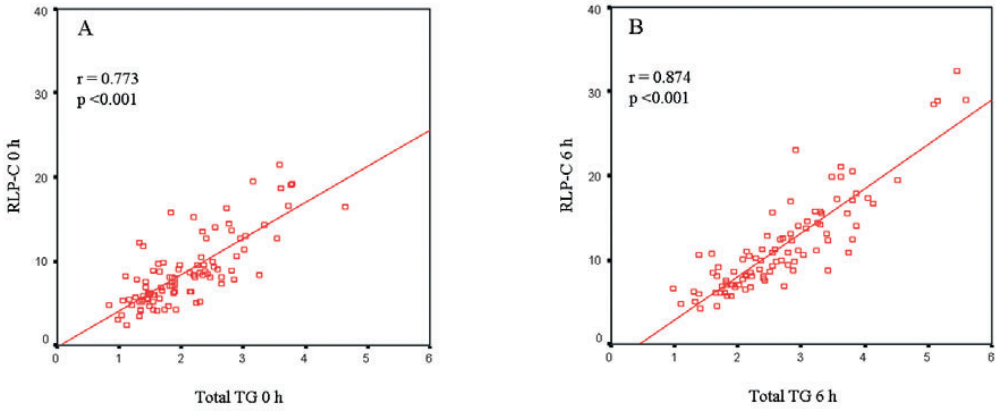


Figure 12 A-B. Correlations among the cholesterol content of remnant lipoprotein particles (RLP-C) and total triglycerides (TGs) in fasting (A) and in fed (B) state.

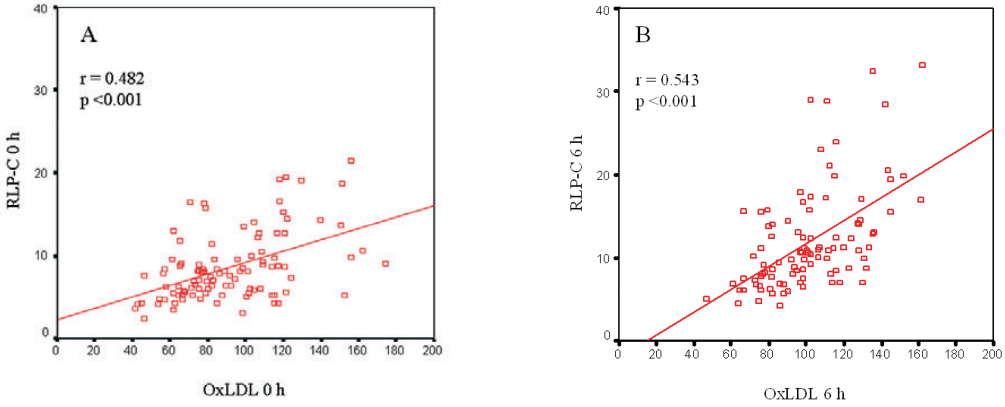


Figure 13 A-B. Correlations among the cholesterol content of remnant lipoprotein particles (RLP-C) and oxidized LDL (oxLDL) in fasting (A) and in fed (B) state.

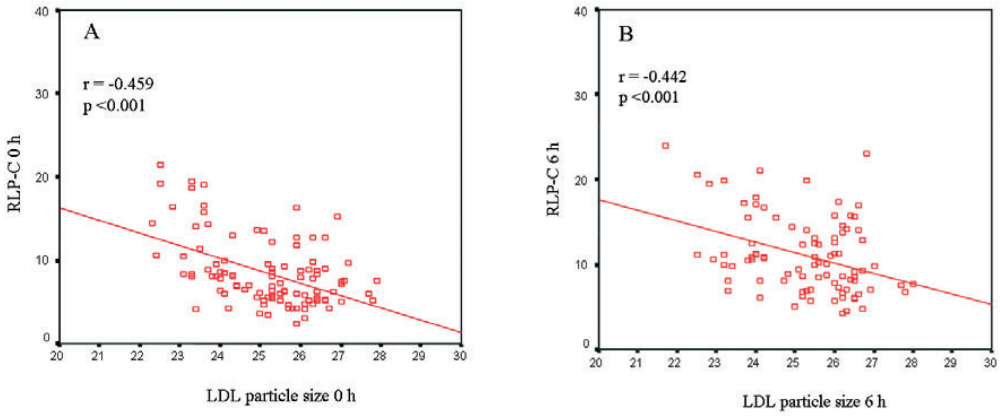


Figure 14 A-B. Correlations among the cholesterol content of remnant lipoprotein particles (RLP-C) and LDL particle size in fasting (A) and in fed (B) state.

6.3.4. Correlation between postprandial lipemia, severity and extent of CAD, and carotid IMT

The concentration of TGs in plasma in Sf>400 or in Sf 12-400 lipoprotein fractions was neither correlated with carotid IMT nor severity and extent of coronary atherosclerosis at 6 hours after oral fat intake or at baseline. Fasting or postprandial values of apoB-48 and apoB-100 in Sf >400 or in Sf 12-400 lipid fractions had no significant association with coronary or carotid atherosclerosis, except the concentration of apoB-48 in Sf 12-400 fraction, which had a weak positive relationship with global atheroma burden index ($r=0.241$, $p<0.05$).

6.3.5. OxLDL, severity and extent of CAD, and carotid IMT

In univariate analysis the concentration of oxLDL in either fasting or in postprandial state did not correlate with global atheroma burden index or carotid IMT.

In multivariate analyses, however, oxLDL was a determinant of global atheroma burden index at postprandial state but not at fasting state ($p=0.042$ versus $p=0.382$, respectively). In addition, age ($p=0.018$), female gender ($p=0.002$), current smoking ($p=0.018$), and to a lesser degree abnormal glucose regulation ($p=0.092$), were predictors of global atheroma burden index. Hypertension, total TG levels, and LDL and HDL cholesterols did not reach significance in the models.

Furthermore, as indicated above, in univariate analysis, TGs showed a strong relationship with RLP-C both in the fasting and in the fed state. Consequently, these variables were not forced into the same model. The results in multivariate regression analyses were, however, similar when replacing TGs with RLP-C.

6.4. Insulin resistance and coronary and carotid atherosclerosis (Study IV)

6.4.1. IR, biochemical variables, and carotid IMT

The study subjects were categorized into three groups. Non-diabetic subjects were divided into group 1 (n=41) with HOMA IR <1.8 (the median value) and group 2 (n=42) with HOMA IR ≥1.8. Diabetic subjects comprised group 3 (n=24).

Clinical, biochemical, and other study variables of the study groups are presented in Table 5. Patients in groups 2 and 3 had higher body mass index, waist circumference, and were more likely to be hypertensive compared with group 1. Total cholesterol, LDL cholesterol, and apoB levels were similar among the three groups. Subjects in groups 2 and 3 had lower HDL cholesterol and apoA-I levels than subjects in group 1. The TGs did not differ significantly between the groups. LDL particle size was smaller in the group 3 compared with group 1. Carotid IMT did not reach statistical significance across the study groups.

Table 5. Clinical, biochemical characteristics and other study variables (n=107).

	Group 1 HOMA IR <1.8 (n=41)	Group 2 HOMA IR ≥1.8 (n=42)	Group 3 Diabetic subjects (n=24)	p*
Age (years)	60 (56-64)	63 (55-65)	58 (52-66)	0.591
Male/Female	25/16 (61/39)	35/7 (83/17)	20/4 (83/17)	0.035
Body mass index (kg/m^2)	25.7 (23.6-27.4)	27.9 (25.7-30.0)	28.3 (27.2-31.6)	<0.001
Waist cirumference (cm)	98±10	105±11	108±9	<0.001
Current or former smoker	19 (46)	27 (64)	14 (58)	0.250
Hypertension	16 (39)	28 (67)	18 (75)	0.006
Previous myocardial infarction	13 (32)	18 (43)	7 (29)	0.434
Previous cerebrovascular disease	1 (2)	3 (7)	0 (0)	0.290
Total cholesterol (mmol/L)	5.74±1.18	5.66±1.27	5.48±1.43	0.728
HDL cholesterol (mmol/L)	1.45±0.32	1.28±0.25	1.22±0.27	0.003
LDL cholesterol (mmol/L)	3.58±1.00	3.57±0.99	3.11±0.89	0.128
Triglycerides (mmol/L)	1.66 (1.23-2.20)	1.99 (1.44-2.36)	1.82 (1.44-3.76)	0.058
LDL particle size (nm)	26.0 (25.6-26.5)	25.5 (24.4-26.3)	24.7 (24.0-26.4)	0.026
ApoA-I (mmol/L)	3.70 (3.26-4.18)	3.24 (3.03-3.82)	3.31 (2.87-3.83)	0.016
ApoA-II (mmol/L)	0.93 (0.84-1.11)	0.93 (0.81-1.14)	0.84 (0.72-1.01)	0.070
ApoB (mmol/L)	3.02±0.74	3.28±0.84	3.28±1.05	0.406
LpA-I (mmol/L)	1.37±0.44	1.22±0.37	1.24±0.26	0.237
LpA-I/A-II (mmol/L)	2.38±0.50	2.20±0.43	2.04±0.45	0.018
Fasting plasma glucose (mmol/L)	5.2 (4.9-5.6)	5.4 (5.2-5.7)	7.1 (6.5-7.9)	<0.001
1-hour plasma glucose (mmol/L)	8.2 (6.0-10.4)	9.2 (7.3-10.1)	12.7 (11.1-15.6)	<0.001
2-hour plasma glucose (mmol/L)	6.1 (5.1-7.4)	6.6 (4.9-7.9)	11.8 (7.8-15.1)	<0.001
Fasting plasma insulin (mU/L)	4.9 (4.2-6.4)	9.8 (8.3-15.6)	11.9 (6.6-20.9)	<0.001
1-hour plasma insulin (mU/L)	46.7 (28.4-61.4)	64.5 (49.9-109.4)	58.2 (42.1-85.9)	0.001
2-hour plasma insulin (mU/L)	35.8 (25.1-43.7)	52.5 (33.6-84.1)	55.4 (27.8-98.1)	0.004
Mean IMT (mm)	1.00±0.18	1.07±0.18	1.07±0.20	0.198
Maximum IMT (mm)	1.26±0.22	1.35±0.22	1.36±0.26	0.156

Data are presented as means ±SD, medians (interquartile ranges), or as frequencies (%).
*p-values from analysis of variance (means of continuous variables) across the three groups.
HOMA IR, the homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ApoA, apolipoprotein A; ApoB, apolipoprotein B; LpA, lipoprotein A; IMT, intima-media thickness.

6.4.2. IR and severity and extent of CAD

The age- and gender-adjusted QCA-derived global indexes for the severity, extent, and overall atheroma burden of CAD were significantly higher in the group 2 than in group 1. Similarly, the global severity, global extent, and global atheroma burden indexes were higher in the group 3 compared with group 1 (Table 6).

We found heterogeneity in associations between IR and CAD indexes according to anatomical location of CAD. Compared with group 1, individuals in the group 2 had more severe and extensive CAD in distal segments only, but not in the left main coronary artery, proximal, or mid coronary segments. Similar results were seen when group 3 was compared with group 1 (Table 6).

Factors independently correlated with global PDS index in multivariate analysis were gender (p=0.002), HOMA IR group (p=0.010), and to a lesser degree waist circumference (p=0.059). Together, these factors explained 29.7% of the severity of CAD. Age, hypertension, smoking status, TGs, and HDL and LDL cholesterols did not reach statistical significance in this model.

Table 6. Quantitative coronary angiography results.

	Group 1 HOMA IR <1.8 (n=41)	Group 2 HOMA IR ≥1.8 (n=42)	p*	Group 3 Diabetic subjects (n=24)	p†
Global indexes					
PDS	24±14	35±11	0.007	35±13	0.027
Extent	11±7	15±5	0.038	16±8	0.090
Atheroma burden	6±4	8±4	0.035	9±6	0.024
PDS index					
Left main coronary artery	0±3	2±7	0.680	4±14	0.123
Proximal segment	12±14	16±12	0.391	15±13	0.587
Mid segment	16±18	23±14	0.227	22±16	0.519
Distal segment	14±10	21±10	0.016	25±14	0.002
Extent index					
Left main coronary artery	0±4	2±10	0.586	6±19	0.147
Proximal segment	13±14	16±12	0.596	16±12	0.495
Mid segment	13±14	20±11	0.085	17±14	0.545
Distal segment	10±7	14±6	0.037	16±9	0.026
Atheroma burden index					
Left main coronary artery	0±1	1±7	0.424	5±22	0.160
Proximal segment	7±8	11±9	0.307	12±14	0.157
Mid segment	8±10	12±10	0.128	12±13	0.362
Distal segment	4±4	6±4	0.125	7±5	0.048

Values are expressed as means ±SD.

*p-values are presented for analysis of covariance between groups 1 and 2 with adjustment for age and gender.

†p-values are presented for analysis of covariance between groups 1 and 3 with adjustment for age and gender.

HOMA IR, the homeostasis model assessment of insulin resistance; PDS, percent diameter stenosis.

6.5. Apolipoprotein E polymorphism and coronary and carotid atherosclerosis (Study V)

6.5.1. ApoE phenotype and biochemical variables

Based on apoE phenotype distribution the study population was divided into two groups; those with apoE3/E3 phenotype (n=51), (apoE3 group), and those with either apoE4/E3 or E4/E4 (n=40), (apoE4 group). Subjects with the phenotypes apoE2/E3 (n=3) and apoE4/E2 (n=2) were excluded from further analysis.

Table 7 shows clinical and biochemical characteristics of all 91 patients by apoE phenotype groups. Total cholesterol and HDL and LDL cholesterols did not differ among the apoE phenotype groups. ApoE4 subjects had higher levels of TGs, RLP-C, apoB, LpA-I/A-II, and Lp(a). The mean LDL particle size was smaller in the apoE4 group than in the apoE3 group. There was no difference in the mean concentration of apoE between the study groups.

Table 7. Clinical and biochemical characteristics by apoE phenotype groups.

Variable	E3 (n=51)	E4 (n=40)	p*
Age (years)	61.2±1.0	58.1±1.1	0.032
Male/female	36/15 (71/29)	29/11 (72/28)	0.841
Body mass index (kg/m^2)	27.3±0.4	27.8±0.6	0.801
Current or former smoker	29 (57)	23 (58)	0.951
Hypertension	30 (59)	22 (55)	0.715
Abnormal glucose regulation	17 (33)	17 (42)	0.370
Previous myocardial infarction	21 (41)	10 (25)	0.106
Previous cerebrovascular disease	1 (2)	3 (8)	0.201
Total cholesterol (mmol/L)	5.54±0.17	5.96±0.20	0.125
Triglycerides (mmol/L)	1.78±0.11	2.54±0.24	0.006
LDL cholesterol (mmol/L)	3.49±0.13	3.62±0.17	0.680
HDL cholesterol (mmol/L)	1.35±0.04	1.35±0.05	0.789
HDL_2 cholesterol (mmol/L)	0.54±0.04	0.54±0.04	0.804
HDL_3 cholesterol (mmol/L)	0.81±0.03	0.81±0.03	0.924
ApoA-I (mg/dL)	134±3	139±4	0.388
ApoA-II (mg/dL)	36±1	37±1	0.215
ApoB (mg/dL)	115±4	135±6	0.008
LpA-I (mg/dL)	51±2	48±2	0.329
LpA-I/A-II (mg/dL)	83±2	92±3	0.030
Lp(a) (mg/dL)	189±22	270±31	0.041
LDL particle size (nm)	25.8±0.2	25.2±0.2	0.041
Fasting RLP-C (mg/dL)	7.83±0.49	12.68±1.92	0.023
ApoE (µg/mL)	37.5±1.7	35.1±2.3	0.370

Values are given as means ±SEM or frequencies (%).

*p-values are presented for Mann-Whitney U-test (continuous variables) or the chi-square test (categorical variables).

LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoA, apolipoprotein A; ApoB, apolipoprotein B; ApoE, apolipoprotein E; LpA, lipoprotein A; RLP-C, the cholesterol content of remnant lipoprotein particle.

6.5.2. ApoE phenotype, severity and extent of CAD, and carotid IMT

As outlined in Table 8 maximum and mean IMT were higher in the apoE4 group than in the apoE3 group even after adjustment for age and gender. Likewise, the global atheroma burden index was higher in the apoE4 group than in the apoE3 group.

Table 8. Carotid IMT and severity and extent of CAD by apoE phenotype groups.

Variable	E3 (n=51)	E4 (n=40)	p*
Mean IMT (mm)	1.02±0.03	1.07±0.03	0.027
Maximum IMT (mm)	1.29±0.03	1.37±0.03	0.022
Global atheroma burden index	6.60±0.63	8.26±0.73	0.033

Data are presented as means ±SEM.
*p-values are presented for analysis of covariance between apoE phenotype groups with adjustment for age and gender.
IMT, intima-media thickness.

The multivariate regression analyses revealed that, age (p=0.001), gender (p=0.001), apoE4 group (p=0.028), and to a lesser degree, smoking (p=0.075), and hypertension (p=0.091) were significant predictors of maximum IMT. ApoB, LpA-I/A-II, LDL particle size, and abnormal glucose regulation were also included in the model, but they did not reach individual significance. The model accounted for 26.6% of the variation of maximum IMT. However, when Lp(a) was added in this model, the impact of apoE4 group was of borderline significance (p=0.060). When only age, gender, and Lp(a) were introduced in the multivariate regression model, Lp(a) was a significant determinant of mean IMT (p=0.012). The results were essentially comparable when mean IMT instead of maximum IMT was used as dependent variable.

Gender (p=0.001) and apoE4 group (p=0.037) were the main determinants that explained 19.4% of variation of global atheroma burden index. When adding Lp(a) in this model, as well, apoE4 group was still related with borderline significance to the global atheroma burden index (p=0.063).

In bivariate correlation analysis apoB was correlated with TGs (r=0.683, p<0.0001) and LDL cholesterol (r=0.608, p<0.0001), respectively. TGs showed a strong relationship with fasting RLP-C levels (r=0.814, p<0.0001). Therefore these variables were not forced into the same model. The results in multivariate regression analyses were, however, similar when replacing apoB with TGs, LDL cholesterol, or RLP-C levels (data not shown).

Further, in bivariate correlation analysis, the concentration of apoE was not related to global atheroma burden index, maximum or mean IMT neither in apoE3 group nor in apoE4 group (data not shown). The absence of an association of the apoE concentration with coronary or carotid atherosclerosis was also seen in multivariate regression analysis (data not shown).

7. DISCUSSION

7.1. General view

This thesis includes a representative sample of 108 previously non-intervened patients, who underwent elective coronary angiography for clinical suspicion of CAD at the Helsinki University Central Hospital. A wide array of disease severity was represented, ranging from angiographically normal coronaries to widespread CAD requiring bypass surgery.

The majority of our patients had several risk factors for CAD, symptoms that suggested angina of functional class II or more, and a positive exercise test result. In addition, a large percentage of participants was taking aspirin, β blockers, and lipid-lowering drugs at baseline. These factors and the relatively small sample size may have overshadowed the relationships between cardiovascular risk factors and the severity and extent of coronary and carotid artery atherosclerosis.

The lack of a control group should be mentioned. However, inclusion of a reference population free from CAD would be unethical, because of the invasive nature of the methods utilized in our study. Furthermore, this study has by nature a cross-sectional design; thus, it is not possible to draw definite conclusions regarding independent risk factors for CAD. A follow-up study is required for such conclusions.

7.2. Methodological aspects

7.2.1. Quantitative coronary angiography

Computer-based analysis of coronary angiograms was used for measuring the severity and extent of coronary atherosclerosis. Invasive coronary angiography is considered the gold standard for assessment of coronary artery stenosis. Even at its best, an angiographic image is a two-dimensional profile of a coronary artery providing only indirect information on pathology within the vessel wall. Moreover, reliance on coronary angiography as a valid measure of severity and extent of coronary atherosclerosis may lead to a lack of accuracy and reproducibility.

Although QCA analysis is a major step forward compared to visual analysis of the coronary angiograms, currently, the most reliably data on severity and extent of CAD would be that obtained by IVUS. Especially in the evaluation of early atherosclerotic changes, IVUS is superior to coronary angiography by providing accurate images of arterial wall. Limitations of IVUS, on the other hand, include difficulty in imaging small, distal coronary segments, inability to cross severe lesions, and suboptimal characterization of plaque components (Topol et al. 1995). Because the strengths and limitations of QCA and IVUS, respectively, are largely complementary, a combination of these two techniques would be optimal in comprehensive evaluation of the severity and extent of CAD.

Conventional catheter-driven coronary angiography, while having demonstrated efficacy, also has persistent small but definite risks, as a result of its invasiveness and requirements for radiation exposure and administration of potentially nephrotoxic contrast agent. Thus, the development of a robust, noninvasive test for defining coronary artery anatomy would be highly desirable.

Noninvasive electron beam computed tomography (EBCT) has a unique combination of high spatial and temporal resolutions, allowing visualization of small lesions. Further, the electrocardiographic triggering allows image acquisition during the slow portion of coronary motion. Therefore EBCT appears well suited to cardiac imaging. In clinical practice, EBCT has been widely used for detecting and quantifying coronary artery calcifications (Budoff et al. 2003). Though coronary artery calcification scores correlate well with the total atherosclerotic burden (Agatston et al. 1994) and strongly predict future cardiac event (Arad et al. 2000), the amount of coronary artery calcification does not correlate well with the stenosis severity of a given lesion (Mautner et al. 1994). Three-dimensional contrast-enhanced EBCT-based angiography has emerged as a technology with the potential for obtaining essentially non-invasive coronary arteriograms. Summary data demonstrate an overall sensitivity of 87% and specificity of 91% for this modality. The main limitations of EBCT-based angiography are the relative inability to visualize distal arteries and collaterals (Budoff et al. 2003).

Coronary magnetic resonance angiography (MRA) is a particularly attractive imaging modality without any invasive action or ionizing radiation. The first results in 1993 revealed a sensitivity of 90% and a specificity of 92% for two-dimensional coronary MRA as compared with conventional angiography (Manning et al. 1993). Despite initial encouraging results and the substantial progress of technical acquisition protocols, reported sensitivities and specificities in detecting CAD still demonstrate a wide variation, and a considerable percentage of segments suffer from degraded image quality (Pundziute et al. 2006).

Multislice computed tomography (MSCT) has emerged as an extremely rapidly developing non-invasive cardiac imaging modality. The diagnostic accuracy has been significantly improved with the recent development of 64-slice scanners. Available data using 64-slice MSCT in comparison with invasive coronary angiography demonstrate sensitivities of 93-99% and specificities of 95-97%, making MSCT the most promising non-invasive imaging modality for the anatomical evaluation of CAD (Pundziute et al. 2006). Still, several important limitations exist. Firstly, the effective radiation dose of 64-slice MSCT scanner is substantially higher (14 mSV) than that of conventional coronary angiography (6 mSv) (Zanzonico et al. 2006). Secondly, although the amount of injected iodinated contrast is decreasing with the newer scanner generations, the use of contrast limits examination of patients with impaired renal function. Thirdly, despite improved temporal resolution in 64-slice scanners, β blockers are still preferable in patients with a heart rate above 70 beats per minutes in order to obtain the best results (Pundziute et al. 2006).

7.2.2. Ultrasonographic measurement of carotid IMT

Atherosclerosis is viewed as a disorder that is restricted to the intima layer of the arterial vessel wall. So far, an ultrasound image cannot discriminate between the intima and media layers of the vessel wall. Thus, an increased IMT may reflect increases in either intima or media thickening, or a combination of both.

Different techniques to measure IMT and reproducibility of results have been thoroughly reviewed by Kanters et al. (1997). Overall the analogy of the FW IMT with histology is more accurate than that of the NW (Wong et al. 1993). Even when the NW IMT is well visualized, its measurement is gain-dependent. However, when gain settings are standardized, the error is systematic and will not bias associations. Moreover, combining NW and FW measurements together seems to reduce the variability and thus increase the reliability of results (Kanters et al. 1997).

The multiple-sites measurements, which were used in our study, usually consist of measuring IMT in the near and far walls of the three main segments of extracranial carotid arteries (common carotid artery, carotid bifurcation, and internal carotid artery) on both sides. By incorporating measurements of IMT in carotid bifurcation and internal carotid artery, early manifestations of carotid atherosclerosis will be taken into account. It is well known that atherosclerosis tends to develop in an asymmetric manner. When the interest is in assessment of atherosclerosis, standardized imaging at different angles and at several sites and calculating the common mean values from these measurements most likely increase the likelihood of capturing all relevant information. More importantly, multiple-sites measurements have given results corresponding more closely with CAD than measurements performed at individual sites (Kanters et al. 1997).

A pooled analysis and evaluation of prospective trials using IMT measurements as surrogate end points of CAD (Bots et al. 2003) recommends the mean of maximum IMT measurements from several sites at the carotid artery as a primary outcome measure, and both NW and FW sonographic measurements, to reduce measurement error, increase precision, and estimate the severity and extent of the carotid atherosclerosis in a reliable way. Thus, our measurements of IMT are in line with the recommendations.

Traditionally, the burden of carotid atherosclerosis has been assessed by measurement of IMT. However, ultrasound scanning can be used to measure aspects of carotid morphology beyond IMT, such as plaque evaluation. In theory, this approach has merit; but, in practice, there has not been a clear cut-off point above which an atherosclerotic carotid plaque can be defined. Recently, the 2004 IMT consensus conference (Touboul et al. 2004) defined plaque as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness ≥1.5 mm as measured from the media-adventitia interface to the intima-lumen interface. Notably, the power of carotid and femoral ultrasound scanning results for predicting cardiovascular mortality has been shown to greatly increase if presence, number, and thickness of plaques are evaluated together with IMT (Griffin et al. 2002).

7.3. Carotid atherosclerosis in relation to coronary atherosclerosis (Study I)

In accordance with previous quantitative imaging studies (Herrington et al. 1994, Blankenhorn et al. 1993), we found that there is a significant relationship between ultrasonically determined carotid IMT and coronary atherosclerosis expressed as quantitative angiographic indexes of severity, extent, and overall atheroma burden of CAD. Herrington et al. (1994), who examined 86 patients with the B-mode score (mean of the maximum IMT at 12 sites) and quantitative coronary angiography (PDS), found a correlation coefficient of r=0.27. Further, in a report from the Cholesterol Lowering Atherosclerosis (CLAS) study, Blankenhorn et al. (1993), demonstrated that carotid artery IMT is significantly correlated with carotid angiographic vessel edge roughness (r=0.31).

There is a significant association between IMT and CAD but, although using refined computer-assisted scoring, the correlation coefficient remains rather moderate suggesting that there are different factors influencing on the carotid and coronary arteries. It has to be acknowledged that the carotid atherosclerotic process is as individual as any coronary segment process; thus, the link between IMT and CAD has great variation.

Epidemiologic data have shown significantly increased cardiovascular event risk among those with IMT of 1 mm or greater (Crouse 2001). In agreement, we found a sharp increase in CAD severity in subjects with IMTs in quartiles higher than the first, corresponding to maximum IMT of 1.16 mm and mean IMT of 0.91 mm or more.

This study is, to our knowledge, the first to show that associations among maximum and mean IMT values, respectively, and quantitative angiographic indexes for proximal, mid, and distal coronary segments differed. Carotid disease was a weaker predictor of CAD in the left main coronary artery and in proximal segments of coronary tree than in mid and distal segments. This finding is intriguing and against conventional wisdom that CAD primarily affects proximal segments, but requires further confirmation.

The relationship we found in this study supports the hypothesis that increased IMT is not only associated with coronary atherosclerotic burden, but that it carries independent information as well. As an indicator of cardiovascular disease and providing a graded measure of vascular damage, carotid IMT may serve as a screening test for identifying patients who would benefit from aggressive diagnostic, therapeutic, and preventive (Greenland et al. 2001) measures. On the other hand, the correlation between carotid IMT and severity and extent of CAD as assessed by QCA is too weak to allow utlilization of IMT as a "gatekeeper" for coronary angiography.

7.4. Determinants of coronary atherosclerosis

7.4.1. PON-1 activity and concentration (Study II)

We detected a significant relationship between PON1 activity and concentration and coronary atherosclerosis expressed as quantitative angiographic indexes of severity, extent, and overall atheroma burden of CAD.

Multiple studies have examined the association between PON1 polymorphisms alone and CAD. A recent meta-analysis using all 43 available studies of the PON1 polymorphisms involving 11 212 CAD cases and 12 786 controls suggested that the link between PON1 polymorphism and CAD is at best weak (Wheeler et al. 2004). However, the vast majority of the studies have not assessed the quality of PON1, i.e. its activity and concentration in the serum.

There is, so far, only limited information available about the association between directly measured PON1 activity and concentration and angiographically proven CAD. Although the difference did not reach statistical significance, Azarsiz et al. (2003) found that PON1 activity of patients with CAD (n=68) was lower than of patients without CAD (n=33) and controls (n=24). Further, Mackness and co-workers (2001) showed that PON1 activity and PON1 concentrations were lower in subjects with CAD than in control subjects. Notably, the result was independent of the PON1 genotype and the authors concluded that the quality of the PON1 enzyme is a more important factor in CAD than is the PON1 gene.

So far, QCA has been applied in only one study comparing PON1 polymorphisms and angiographic severity and extent of CAD (Chen et al. 2003a). In a report from the Women´s Ischemia Syndrome Evaluation (WISE) study including 711 women, Chen et al. (2003a) did not find any significant association between the PON polymorphisms and stenosis severity in either white or black women. However, when patients with significant CAD (≥50% stenosis) were stratified into groups with one-, two-, or three-vessel CAD, significant associations were noted between PON polymorphisms and the number of diseased vessels in whites but not in blacks. In contrast to our study, the study of Chen et al. (2003a) comprised only women and data on PON1 activity and concentration were not available.

This study is, to our knowledge, the first to present data on PON1 activity and concentration in patients with CAD measured both by visual interpretation and by refined computer-assisted scoring of coronary angiograms. Our results show that PON1 activity and concentration were lower in patients with significant CAD. Moreover, PON1 activity and concentration were significantly associated with the indexes for global severity, extent, and atheroma burden of CAD. We found a stepwise decrease in these QCA-derived indexes across the tertiles of PON1 activity and concentration. Subjects in the lowest tertile of PON1 activity and concentration, respectively, had a more severe and extensive disease than subjects in the highest tertile.

Activity and concentration of PON1 can vary up to 40-fold in human populations (Mueller et al. 1983, Richter et al. 1999). Although PON1 activity and con-

centration are determined genetically, various kinds of physiological and pathological states, such as diet, life-style, environmental chemicals, and drugs can modulate PON1 levels. PON1 decreases in older people, during pregnancy, and menopause (Mackness et al. 2002). Low serum PON1 activity independent of genotype has been reported in diseases associated with accelerated atherogenesis, such as DM, hypercholesterolemia, and renal failure (Mackness et al. 1991, Abbott et al. 1995, Hasselwander et al. 1998). Degraded cooking oil and an atherogenic diet have been reported to lower serum PON1 in humans (Sutherland et al. 1999). Dietary polyphenols (present in wine, tea, fruit juice) increase PON1 activity, as does moderate alcohol intake (van der Gaag et al. 1999, Kaplan et al. 2001). Vitamin C and E intake is associated with increased PON1 activity (Jarvik et al. 2002). However, another study in which vitamin E was given to volunteers showed no change in PON1 activity (Arrol et al. 2000). Smoking is known to decrease serum PON1 activity (James et al. 2000). Recent evidence shows that exposure to environmental chemicals can inhibit PON1 activity (Sozmen et al. 2002, Serhatlioglu et al. 2003). Understandably most of the interest in pharmacological effects on PON1 activity has, thus far, been in effects of lipid-lowering drugs. Some of these studies (Aviram et al. 1998b, Tomas et al. 2000, Paragh et al. 2000), but not all (Durrington et al. 1998, Turay et al. 2000, Balogh et al. 2001) suggest an effect of statins and fibrates in raising PON1 activity. Lately, Hong et al. (2006) demonstrated, in hypercholesterolemic rabbits, that probucol, a cholesterol-lowering drug with antioxidative property, increases PON1 serum level as well as mRNA expression of PON1 in hepatocytes. Of note, no correlation was observed between PON1 and HDL cholesterol level. Additionally, a large case-control study indicated that aspirin users have higher serum activities but also concentrations of PON1 (Blatter Garin et al. 2003). Such effect may be due to the anti-inflammatory effect of aspirin, as serum PON1 levels are reduced during the inflammatory response; alternatively, aspirin may act as an antioxidant (Blatter Garin et al. 2003).

In human serum most if not all of the paraoxonase activity is associated with HDL. Paraoxonase is present in a distinct HDL subspecies containing apoA-I and clusterin or apo J (Blatter et al. 1993). La Du et al. (1989) demonstrated that it is extremely difficult to remove apoA-I from PON1 during purification from human serum, which has led to the suggestion that apoA-I and PON1 are closely associated. Our results are in line with population studies that have shown a statistical association of PON1 activity with HDL cholesterol, apoA-I, and apoA-II (Boman et al. 1980, La Du et al. 1989, Abbott et al. 1995).

Interestingly, our results in the multivariable analyses indicate that PON1 activity is a significant determinant of severity of CAD independently of HDL cholesterol, apoA-I, and apoA-II. This suggests that PON1 activity has an important role in the pathogenesis of coronary atherosclerosis. Our findings demonstrate the relevance of PON1 activity and concentration for describing associations between atherosclerotic disease and the enzyme. More importantly, the study illustrates how the protective role of HDL could be modulated by its components such that equivalent serum

concentrations of HDL cholesterol may not equate with an equivalent, potential protective capacity. The recent disappointing results with the CETP antagonist torcetrapib suggest that not all HDL cholesterol elevating therapy may confer protection against cardiovascular disease. Additionally, in contrast with other cholesterol-lowering drugs, probucol is known to decrease HDL cholesterol of around 20% to 30 %. The clinical significance of this observation is unclear, although some investigations suggest a beneficial effect in enhancing reverse cholesterol transport (Sawayama et al. 2002). In fact, probucol has been shown to retard the progression of carotid IMT (Sawayama et al. 2002) and to inhibit restenosis after percutaneous coronary intervention (Tardif et al. 2003). Nevertheless, clinical use of probucol has been limited because of its effects on the QT-interval (Hong et al. 2006).

Although significant advances have been made in understanding the PON family of proteins, there are still unresolved issues. Firstly, PON1, PON2, and PON3 have all shown to protect against LDL oxidation, but their precise physiological function remains unknown. Their different enzymatic activities, localization, and regulation suggest that they may possess different functions (Ng et al. 2005). Secondly, PON1 activity can conveniently be directly measured in the serum or plasma. Opinions differ today, however, which PON1 substrates best represent the "protective" role of PON1 in connection with cardiovascular disease. Thirdly, additional information is required particularly about nutritional and pharmacological effects on serum PON1 activity (La Du 2003).

7.4.2. Postprandial lipemia, oxLDL, and LDL particle size (Study III)

The present data demonstrate, for the first time in patients with clinically suspected CAD referred for coronary angiography, that circulating oxLDL significantly increases postprandially. There was a highly significant positive correlation between postprandial TRLs and postprandial oxLDL suggesting that the postprandial state creates oxidative stress. In this cohort, multivariate analysis revealed that postprandial oxLDL independently predicted the severity and extent of coronary atherosclerosis determined by QCA. This emphasizes the pivotal role of LDL oxidation in the development of atherosclerosis even after inclusion of conventional CAD risk factors.

Small, dense LDLs are more susceptible to oxidation than native LDL particles (de Graaf et al. 1991, Diwadkar et al. 1999). It has recently been reported that, in a cohort of patients with myocardial infarction, the marked accumulation of TRLs accelerated the remodeling of LDL particles toward smaller, denser particles (Koba et al. 2005). In contrast to Koba et al. (2005), we did not find any significant changes in LDL particle size. Likewise, we have previously reported that LDL particle size did not change during an 8-h postprandial period in patients with type 2 DM (Vakkilainen et al. 2002b). We found, however, an inverse relation between LDL particle size and TRLs postprandially. This is in agreement with the well-established inverse correlation of serum TGs with LDL particle size (Packard et al. 2000).

The role of TGs as an independent risk factor of CAD has been debated for decades. A meta-analysis of 29 prospective population-based studies indicated highly significant associations between TG values and CHD risk. However, these associations depended considerably on levels of established risk factors, especially HDL cholesterol (Sarwar et al. 2007). In the Honolulu Heart Study, RLP levels did not provide additional information about CHD incidence as compared to concentration of total TGs (Imke et al. 2005). They speculated that their result could be attributed to the strong correlation between RLP levels and total TGs. Similarly, we found a strong correlation between RLP-C and total TGs both in the fasting and in the fed state. In the present study TGs, apoB-48, apoB-100, and RLP-C at fasting or at postprandial state did not associate with the severity and extent of coronary atherosclerosis determined by QCA. In addition, in multivariate analysis neither fasting nor postprandial TGs were independent determinants of global atheroma burden index.

Traditionally TRLs have been isolated by density-gradient ultracentrifugation. Due to the fact that this method is complex and time-consuming a new technique to isolate remnant lipoproteins was developed using an immunoaffinity mixed gel containing anti-apoA-I and anti-apoB-100 monoclonal antibodies (Nakajima et al. 1993). We employed a RLP assay to quantify the postprandial increase of TRLs in order to gain more information on atherogenicity. The correlations between TGs, apoB-48, and apoB-100 in TRLs isolated by density-gradient ultracentrifugation and RLPs in fasting and postprandial state were highly significant. Thus, the excellent correlations especially postprandially suggest that the RLP assay is a good supplement to the tedious method of ultracentrifugation.

Holvoet and his colleagues (2001) were the first to clearly demonstrate that patients with angiographically proven CAD had significantly elevated plasma levels of oxLDL measured in the fasting state. Recently, Meisinger et al. (2005) concluded, in a prospective study comprising men, that oxLDL was a stronger predictor of risk than standard lipid variables and other traditional risk factors. We observed a significant increase of circulating oxLDL after the fatty meal. Interestingly, postprandial oxLDL was a significant predictor of severity and extent of coronary atherosclerosis using refined computer-assisted scoring of coronary angiograms. Our findings are in agreement with data suggesting that oxidative stress is increased postprandially (Diwadkar et al. 1999, Ceriello et al. 2004). However, to the best of our knowledge, this has not been previously reported at postprandial state in CAD patients rigorously assessed for coronary atherosclerosis by QCA. Furthermore, circulating oxLDL and small, dense LDL particles were associated with remnants of TRLs emphasizing the atherogenic nature of postprandial state.

Lecitin-like oxLDL receptor-1 (LOX-1) is a vascular endothelial receptor for ox-LDL (Sawamura et al. 1997). Shin et al. (2004) reported that RLPs caused LOX-1 protein expression, and that increased production of both superoxide and cytokines and enhanced DNA fragmentation by RLPs was significantly inhibited by monoclonal antibody for LOX-1 receptor. They showed that RLPs caused a significant increase in nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase-

dependent superoxide production via activated LOX-1 receptors. These findings strongly suggest that LOX-1 is a receptor for RLP as well as oxLDL on endothelial cells.

Although emerging evidence utilizing immunological techniques suggests an impact of circulating oxLDL on CAD, there are some caveats concerning the extent of modification and reproducibility of the reference oxLDL preparations that are used as standards for these assays. There is only one epitope measured in these assays whereas potentially many epitopes may exist on any one individual LDL, and the fact that different populations of oxLDL may exist. The only way to be certain of the amount of a particular epitope that is quantitated is to ensure that the reference standard has the same extent of oxidation on LDL as the measured LDL. The quantitation of oxLDL will be improved when the epitopes are better delineated and the reference standard can be a stable pure preparation of the epitope (Tsimikas et al. 2001).

7.4.3. Insulin resistance (Study IV)

This study indicated that IR was associated with coronary atherosclerosis expressed as quantitative angiographic indexes of severity, extent, and overall atheroma burden of CAD. In addition, IR seemed to be a stronger predictor of coronary atherosclerosis in the distal parts of the coronary tree than in the proximal and mid parts. In the multivariate analysis IR was a significant predictor of the severity of CAD.

To our knowledge, only two studies have investigated the relationship between IR and coronary atherosclerosis utilizing quantitative imaging techniques. Korpilahti et al. (1998) showed that, in a 5-year follow-up study of 228 coronary artery bypass surgery patients, IR determined as the insulin sensitivity index was associated with both progression of pre-operative atherosclerotic lesions and development of new lesions. In studying a cohort of 95 non-diabetic Japanese subjects with CAD verified with semi-quantitative coronary angiography, Tsuchihashi et al. (1999) found that hyperinsulinemia was an important risk factor for CAD explaining the severity of coronary atherosclerosis.

Our study confirms previous positive findings (Bressler et al. 1996, Sasso et al. 2004, Yanase et al. 2004) and extends them as: 1) our study population included men and women; 2) was across a wide array of disease severity, ranging from angiographically normal coronaries to widespread CAD requiring bypass surgery; 3) our study comprised CAD patients with normal and abnormal responses to a 75-g oral glucose tolerance test; 4) we used refined computer-assisted scoring of CAD; and 5) we found a positive relationship between age- and gender-adjusted severity of IR and coronary atherosclerosis. In fact, non-diabetic subjects with more severe degree of IR, i.e. HOMA IR score above the upper median level of 1.8, were comparable with diabetic subjects in terms of severity and extent of CAD.

Consistent with other reports we found that, besides having a more severe and extensive CAD, type 2 diabetic patients more frequently had lesions located on distal arteries than non-diabetic patients (Henry et al. 1997, Thomas et al. 2002). In addition, our data show that even non-diabetic subjects with a more severe degree

of IR had a more distal type of CAD compared with individuals with a lower degree of IR. To the best of our knowledge, this has not been previously published.

The process of lesion formation in CAD is complex. Endothelial dysfunction, an imbalance between endothelium-derived vasodilative (e.g., nitric oxide) and vasoconstrictive (e.g., endotelin-1) factors, is regarded as an early pivotal event in atherogenesis and cardiovascular disease and is closely linked to obesity and IR (Steinberg et al. 1996). In addition, obesity, IR, and endothelial dysfunction coexist and they can all be identified in individuals with type 2 DM as well as in various groups at risk for type 2 DM, such as in individuals with impaired glucose tolerance, family history of type 2 DM, hypertension, and dyslipidemia (Caballero 2003). Recently, Prior and co-workers (2005) demonstrated that, even in the absence of traditional coronary risk factors, the greatest loss in nitric oxide-mediated, endothelium-dependent flow occurred when IR was the only abnormality, and this seemed to worsen progressively with more severe states of IR.

During the development of coronary atherosclerosis compensatory arterial enlargement preserves the intraluminal space and delays clinically important lumen stenosis (Stiel et al. 1989, Glagov et al. 1987). The mechanism for compensatory enlargement is thought to be predominantly mediated by the vascular endothelium in response to changes in the hemodynamic, shear stress, and humoral changes in the vessel lumen (Gibbons et al. 1994). This coronary artery remodeling has been shown to be inadequate or negative in diabetic patients (Vavuranakis et al. 1997, Kornowski et al. 1998). Arterial remodeling, however, is not a homogeneous process within the vessel wall. Interestingly, Nishioka et al. (2001) reported that, despite a similar degree of luminal narrowings, the proximal coronary segments showed more prominent compensatory enlargement than the distal arterial segments. Recently, Fischer et al. (2005) found that, in families with myocardial infarction, coronary artery stenoses are particularly heritable at proximal localizations, whereas no heritability was found for distal disease. In our study the reason for different impact of IR on anatomic characteristics of coronary atherosclerosis remains unclear, but our results support a regional heterogeneity in remodeling responses.

7.4.4. ApoE polymorphism (Study V)

We demonstrated for the first time that apoE polymorphism contributes similarly to both ultrasonically determined carotid IMT, and coronary atherosclerosis expressed as quantitative index of overall atheroma burden of CAD inside the same population. Patients with apoE4 phenotype had an increased carotid IMT and a more severe and extensive CAD than patients with apoE3 phenotype. These associations were independent of age and gender, which are known to influence both carotid and coronary atherosclerosis.

Multiple studies have examined the association between CAD and apoE polymorphism. A recent meta-analysis based on 48 published reports (21 clinical and 27 angiographically confirmed CAD) fully supports the impact of the ε4 allele as a significant risk factor for CAD (Song et al. 2004). The analysis identified a significantly

increased risk for CAD among carriers of the ε4 allele compared with carriers of the ε3 allele. Furthermore, the meta-analysis found little evidence of an association between the ε2 allele and CAD risk.

To our knowledge, only one previous study has examined the association between the apoE polymorphism and CAD severity based on a computer-assisted analysis of coronary angiograms. Our data confirms and expands the results shown by Chen et al. (2003b) who reported a significant relationship between the ε4 allele and coronary artery stenosis measured by QCA. However, unlike our cohort that included gender main streaming, i.e. both males and females, the study of Chen et al. (2003b) comprised only women.

Our data show higher apoB concentrations in patients with apoE4 phenotype. The association of ε4 allele with increased LDL cholesterol and apoB concentrations is a well-established issue (Davignon et al. 1999, Curtiss et al. 2000). Possible explanations to the atherogenicity of the ε4 allele are the high affinity of apoE4 to apoE-binding receptors, thereby leading to increased apoE-mediated cholesterol uptake of liver cells. This increase in the hepatic cholesterol pool has the potential to down-regulate the LDL receptor, resulting in an accumulation of LDL cholesterol and increased risk of atherosclerosis. Further, ε4 allele may be associated with a high intestinal cholesterol absorption efficiency, but studies have yielded conflicting results (Miettinen et al. 1992, Bergmann et al. 2003).

The present study indicates that some of the effects of apoE polymorphism on carotid and coronary atherosclerosis may be mediated by Lp(a), since the concentration of Lp(a) was significantly higher in the apoE4 group than in the apoE3 group. In addition, in the multivariate analyses, including Lp(a) in the model almost dropped out the independency of apoE polymorphism. Notably, when age, gender, and Lp(a) were included without apoE phenotype in the multivariate model, Lp(a) remained a significant predictor of carotid IMT.

Little is known about the catabolism of Lp(a). Lp(a) binds weakly to the LDL receptor, and it is therefore unlikely that the LDL receptor is the major determinant of Lp(a) degradation (Armstrong et al. 1990, Kostner 1993). It has been suggested, that LDL receptor-related protein, which is a multi-ligand receptor that binds apoE-containing remnant lipoproteins, is involved in the metabolism of Lp(a) (März et al. 1993).

Although substantial evidence has accumulated that elevated Lp(a) is an independent risk factor for cardiovascular disease (Danesh et al. 2000) several issues limit the utility of Lp(a) screening. First, the quantitative contribution of elevated Lp(a) to CHD risk beyond the major risk factors is uncertain. Second, Lp(a) levels vary widely among different racial groups. They are higher in African Americans than in Caucasians, but an increased risk for CHD associated with higher Lp(a) levels in African Americans has not been documented (Moliterno et al. 1995). Third, the major obstacles to the progress of Lp(a) research have been issues related to the measurement of Lp(a) and the absence of an international standard. Current methods of measuring Lp(a) levels include electrophoresis, electroimmunodiffusion, electroimmunoassay, radioimmunoassay, immunoturbimetric assay, and ELISA (Danesh et al.

2000). Finally, serum Lp(a) is relatively resistant to therapeutic lowering. Among currently available drugs, only nicotinic acid reduces Lp(a) concentrations, and only moderately (Angelin 1997). To date, no clinical trials have shown that lowering Lp(a) levels decreases CHD risk (Fruchart et al. 2004).

Higher levels of TRLs, derived from VLDLs and CMs, have been linked to an increased risk of atherosclerosis (Karpe et al. 2001, McNamara et al. 2001). RLPs measured by immunoseparation techniques are often used as a surrogate measure of TRLs. Recently, Devlin et al. (2005) demonstrated that high levels of apo(a)/Lp(a) in vivo, inhibited hepatic clearance of remnants, leading to high plasma levels of RLPs and markedly enhanced atherosclerosis. Whether this function is associated more with the ε4 allele than the ε3 allele is an interesting question, since, in our patients, RLP-C was higher in patients in the apoE4 group than in the apoE3 group. Notably, TGs were also higher in apoE4 carriers than in non-carriers, which is in line with the results of the meta-analysis by Dallongeville et al. (1992).

Still, additional mechanisms may link apoE polymorphism with atherosclerosis. Some studies have suggested that the association of apoE polymorphism with atherosclerosis cannot be totally attributed to variations in total and LDL cholesterols (Eichner et al. 1993, Wilson et al. 1994). In mice, physiological levels of apoE can induce atherosclerosis regression independently of lowering plasma cholesterol levels (Raffai et al. 2005). Another mechanism was recently proposed by Heeren and associates (2004) who reported that the recycling of apoE originating from TRLs was impaired in apoE4 in comparison to apoE3 in human hepatoma cells resulting in decreased cholesterol efflux.

Because of the polymorphic nature of apoE in humans, there are considerable limitations in the current use of apoE genotyping as an assessment of vascular risk. First, the frequency of the ε4 allele differs in populations being higher in northern parts of Europe where CAD is more prevalent (Davignon et al. 1999). Second, the frequency of the ε4 allele has been reported to be age- and gender-related, although the data are inconsistent (Kolovou et al. 2003). Third, there is evidence of several gene-gene interactions involving the apoE locus. For example, plasma TG levels have been found to be simultaneously modulated by variations at the lipoprotein lipase gene and the apoE gene (Davignon et al. 1999). Fourth, coexistence of lifestyle factors can modulate the link between apoE and atherosclerosis. Although the results are controversial (Boerwinkel et al. 1991, Lefevre et al. 1997), the ε4 allele has been associated with increased LDL cholesterol in response to dietary fat (Tikkanen et al. 1990, Ordovas et al. 1995). The association between HDL cholesterol and physical activity may be apoE dependent (Bernstein et al. 2002). In addition, alcohol intake and smoking have shown to influence the effect of apoE (Corella et al. 2001, Humphries et al. 2001). Finally, apoE polymorphism shows its protagonism in the field of pharmacogenetics. In the study by Pedro-Botet et al. (2001), comprising 328 male and female volunteers who participated in a multicenter, double-blind clinical trial and received 10 mg/day of atorvastatin, men carrying the ε2 allele appeared to be more responsive to statin therapy than those carrying the ε4 allele. Notably, this interaction was absent in women. Further, data suggest that apoE polymorphism have

a differential effect on lipids and lipoproteins in postmenopausal women treated with hormone-replacement therapy, with ε2 allele carriers showing the most beneficial response (Ordovas et al. 2002). Estrogens are known to upregulate hepatic LDL receptors, whereas apoE4 induces its downregulation. Therefore, the estrogenic effect may be counteracted by the presence of apoE4 isoform. Conversely, apoE2 upregulates the LDL receptor and may work in syngergy with estrogens to reduce LDL cholesterol levels (Ordovas et al. 2002).

7.5. Determinants of carotid atherosclerosis

7.5.1. PON-1 activity and concentration (Study II)

Only a few studies have examined the association betweeen PON1 activity and concentration and carotid artery disease. Jarvik et al. (2000) reported in a case-control study, that 106 carotid artery disease cases (i.e. >80% internal carotid artery stenosis on angiography) had significantly lower levels of paraoxonase and diazoxonase activity. In multivariate analysis they found that PON1 Q/R192 and PON1 M/L55 genotypes or haplotypes did not predict case-control status unless the activity phenotype was also included. Campo et al. (2004) showed, in a study comprising 208 Sicilian subjects with hypercholesterolemia (i.e. total cholesterol >6 mmol/L), that there was no significant association between PON1 Q/R192 and PON1 M/L55 polymorphisms and ultrasonically measured early carotid atherosclerosis. They did not find any significant correlation between PON1 activity using phenylacetate or paraoxon and carotid IMT. Additionally, Valabhji et al. (2001) found in a case-control study, that 35 patients with type 1 DM had increased carotid IMT, but the PON1 activity using phenylacetate as substrate was similar in diabetic and non-diabetic subjects. Likewise, we could not find a significant association between PON1 activity and concentration and carotid IMT.

7.5.2. Postprandial lipemia, oxLDL, and LDL particle size (Study III)

A limited number of studies have investigated the relationship between remnant lipoproteins and carotid atherosclerosis. Karpe et al. (1998) showed, in a study comprising 30 healthy normo- and hypertriglyceridemic middle-aged men, that late postprandial TG levels were associated with carotid IMT. Further, Boquist et al. (1999) found, in a study including 96 healthy 50-year-old men with an apoE3/E3 genotype, that postprandial triglyceridemia was an independent risk factor for early atherosclerosis. Another study conducted in the same cohort showed that, among the major fasting plasma lipids and lipoproteins, common carotid artery IMT was most strongly correlated with RLP-C (Karpe et al. 2001).

To the best of our knowledge, there are no previous studies on postpandial lipemia and carotid IMT in males and females with clinically suspected CAD. Our

data argue against an important role for postprandial lipemia in the predisposition of carotid atherosclerosis. However, one of the main problems of interpreting the associations of TGs with atherosclerosis is the biochemical and metabolic heterogeneity of TRLs.

Data on associations between carotid IMT and circulating oxLDL or autoantibodies against oxLDL have given inconsistent results. Susceptibility of LDL to oxidation has been associated to carotid IMT in 30 eastern Finnish men with accelerated 2-year progression of carotid atherosclerosis (Salonen et al. 1992), in a study group of 391 clinically healthy 58-year-old men (Hulthe et al. 2002), as well as in a prospective, observational study with more than 3 years of follow-up in 326 clinically healthy men (Wallenfeldt et al. 2004). In contrast, oxLDL was not related to carotid IMT in a case-control 10-year follow-up study of 91 patients with non-insulin-dependent DM (Uusitupa et al. 1996) or in the large population-based Bruneck study (Mayr et al. 2006). The differences in the stages of atherosclerosis, study designs, and methods of measuring oxLDL may account for these inconsistent findings.

LDL particle size has been associated with carotid IMT in middle-aged healthy subjects (Skoglund-Andersson et al. 1999, Hulthe et al. 2000a). However, LDL particle size was not associated with carotid IMT in patients with hypercholesterolemia (Hulthe et al. 2000b) or with CAD risk among elderly subjects (Mykkänen et al. 1999). Again, the difference in the stage of atherosclerosis and the study design may explain why we were not able to find a significant association between LDL particle size and carotid IMT.

7.5.3. Insulin resistance (Study IV)

The association of IR with carotid IMT has been addressed in several studies, but the results are inconsistent. Suzuki et al. (1996) and Shinozaki et al. (1997) reported that IR, in non-diabetic subjects, was independently and significantly associated with carotid IMT. In contrast, Ishizaka and co-workers (2003) showed that, in a study population including 738 with normal glucose tolerance, 334 with both impaired fasting glucose and impaired glucose tolerance, and 166 diabetic subjects, there was no significant association between HOMA IR and carotid IMT in either the group as a whole or in patients with normal glucose tolerance. In the present study, the results concerning the relationship between HOMA IR and carotid IMT did not reach statistical significance.

7.5.4. ApoE polymorphism (Study V)

Carotid atherosclerosis is considered a surrogate marker for coronary atherosclerosis; thus, measures of IMT by ultrasound provide a quantitative basis for the extent of atherosclerosis. The association between the ε4 allele and an increased carotid IMT has been observed in several studies (Terry et al. 1996, Cattin et al. 1997, Elo-

sua et al. 2004), although others have failed to find such an association (Slooter et al. 2001, Fernandez-Miranda et al. 2004). Most of these studies have been performed in subjects without a relevant atherosclerosis risk, but only two (Terry et al. 1996, Fernandez-Miranda et al. 2004) in individuals with CAD. Our data are consistent with those documented by Terry et al. (1996), who found a significant association of apoE polymorphism with carotid IMT.

8. SUMMARY AND CONCLUSIONS

The main findings and conclusions of the present study can be summarized as follows:

1) In this Finnish cohort of patients undergoing elective coronary angiography, there was a significant relationship between ultrasonically determined carotid IMT and coronary atherosclerosis expressed as quantitative angiographic indexes of the severity, extent, and overall atheroma burden of CAD. We found heterogeneity in associations between IMT and CAD indexes according to anatomical location of CAD. Maximum and mean IMT values, respectively, were correlated with quantitative angiographic indexes for mid and distal segments but not with the proximal segments of coronary vessels.

   Our observations show an association between carotid IMT and the severity and extent of CAD.

2) The values of PON1 activity and concentration, respectively, were lower in subjects with significant CAD and there was a significant relationship between PON1 activity and concentration and CAD assessed by QCA. PON1 acitivity was a significant determinant of severity of CAD independently of HDL cholesterol, apoA-I, and apoA-II. This suggests that PON1 activity has an important role in the pathogenesis of coronary atherosclerosis.

   Our data demonstrate the relevance of PON1 activity and concentration for describing associations between atherosclerotic disease and the enzyme. More importantly, the study illustrates how the protective role of HDL could be modulated by its components such that equivalent serum concentrations of HDL cholesterol may not equate with an equivalent, potential protective capacity.

3) RLP-C in the fasting state is a good marker of postprandial TRLs. Circulating oxLDL increases in CAD patients postprandially. We noticed a highly significant positive correlation between postprandial TRLs and postprandial oxLDL, which suggests that the postprandial state creates oxidative stress. Postprandial oxLDL independently predicted the severity and extent of CAD determined by QCA.

   Our findings emphasize the pivotal role of LDL oxidation in the development of atherosclerosis even after inclusion of conventional CAD risk factors.

4) IR was associated with an increased severity and extent of coronary atherosclerosis and seemed to be a stronger predictor of coronary atherosclerosis in the distal parts of the coronary tree than in the proximal and mid parts. In the multivariate analysis IR was a significant predictor of the severity of CAD.

   Our data suggest that disturbances in glucose metabolism play an important role in pathogenesis of coronary atherosclerosis. In view of our results, it seems appropriate to intensify efforts to identify insulin-resistant individuals so that intensive treatment strategies can be directed to lower their risk of CAD and to delay the onset of type 2 DM.

5) ApoE polymorphism contributes similarly to both ultrasonically determined carotid IMT and coronary atherosclerosis expressed as quantitative index of overall atheroma burden of CAD inside the same population. Patients with the apoE4 phenotype had an increased carotid IMT and a more severe and extensive CAD than patients with the apoE3 phenotype.

   Our results indicate that apoE polymorphism is involved in the susceptibility to both carotid and coronary atherosclerosis.

Future implications for research:

Our findings highlight the complex relationships between traditional and emerging cardiovascular risk factors and atherosclerotic disease burden. Indeed, they shed some new intriguing light on the complexity. Therefore, it would be interesting to confirm these results in a large population of patients with clinically suspected CAD. Moreover, it is highly desirable to increase the clinical understanding of atherosclerotic plaques prone to rupture. The latest technology developed for this purpose is spectral analysis of IVUS radio frequency data. This technique has improved the in vivo tissue characterization in CAD to a degree that it is called virtual histology (VH).

In a further study it would be exciting to identify patients at increased risk of cardiovascular events incorporated in a multivariate score comprising cardiovascular risk factors, data from carotid IMT, coronary angiography, conventional IVUS, and VH IVUS. There has never been a simple answer to a complex diagnostic problem.

9. ACKNOWLEDGEMENTS

This work was carried out at the Department of Medicine, Division of Cardiology, Helsinki University Central Hospital. I am deeply grateful to Professor Markku S. Nieminen, MD, Head of the Division of Cardiology, for introducing me to the present theme, for providing me excellent research facilities, and for his continuous encouraging attitude towards my scientific work.

I have had the great privilege to work under the firm supervision of a distinguished word-class scientist, Professor Marja-Riitta Taskinen, MD. Her expertise, enthusiastic guidance, never-ending patience, and continuous friendly support have been the driving force of this work. I wish to extend my deepest gratitude to my other supervisor, Docent Mikko Syvänne, MD, for his vast scientific knowledge and clinical experience in the field of cardiovascular diseases. His constructive criticism during the review of the manuscripts and thesis has been invaluable.

Professor Timo Strandberg, MD, and Docent Raimo Kettunen, MD, the official reviewers of this thesis, are gratefully acknowledged for their careful evaluation, precious comments, and supportive attitude.

My collaborators are kindly appreciated for their contributions to this thesis. I am deeply indepted to Professor Richard W. James, MD, Takamitsu Nakano, PhD, Docent Matti Jauhiainen, PhD, Docent Riitta M. Salonen, MD, and Docent Kristiina Nyyssönen, PhD for their scientific advice and valuable comments of the manuscripts. I warmly thank Professor Seppo J. Sarna for sharing his expertise in statistics. My sincerest thanks are due to Marjut Varpula, MD, who performed all the IMT scans, and Juhani Kahri, MD, PhD, for friendly guidance and valuable help in preparing the manuscripts.

I am immensely thankful to Gun-Christine Svahn for outstanding help in recruiting the study subjects and for superbly efficient nursing assistance. The laboratory excellence of Hannele Hildén, Helinä Perttunen-Nio, and Sari Nuutinen is gratefully acknowledged. I thank Arja Malkki for skillful reading of the ultrasound scannings.

My special thanks are due to Joachim Stjernvall, MD, who performed most of the coronary angiographies. I am also thankful to Pia Pajunen, MD, PhD, Sakari Mänttäri, MD, and Kati Ylitalo, MD, PhD, for their collaboration and support.

I acknowledge the great help from Markku Ventilä, MSc (Tech.) in situations concerning software problems and Leena Lajunen, MSc, for providing superb library services.

I am grateful to all the patients who volunteered in the study and made this work possible.

I want to thank all my colleagues and the personnel of the Cardiovascular Laboratory, medical wards, and Emergency Units in Meilahti Hospital for their friendship and encouragement.

My sincere gratitude is expressed to Professor Kimmo Kontula, MD, and Professor Vuokko Kinnula, MD, the former and the present Head of the Insitute of Clinical Medicine, Faculty of Medicine, University of Helsinki for giving me the possibility to work as clinical teacher. Thanks are also due to Sirpa Huhtaniitty, Viveka Metsäaho, and Tuulikki Tötterman for educational assistance.

I am indebted to my medical students for critical feedback on clinical issues during all these years. This youthful support has maintained my belief in the future of medicine.

I gratefully acknowledge my dear friend and colleague Magnus Lindroos, MD, PhD, who in the very beginning guided me by hand to the world of science and clinical cardiology. Thank you, Magnus, for years of reliable collaboration.

I extend my sincere thanks to all my relatives and friends, for their endurance and charity, although I have not had enough time for them during the past years. I especially thank Kaj Sahipakka for long-lasting friendship and support. My warm thanks are due to Maggi Bast-Gullberg, Pia Småros-Berndtsson, Anki Sund, and Rose Taponen for sharing many unforgettable moments together particularly in the good old 80ies. Much of my spare time has been devoted to singing, and I am thankful to all members of the Swedish Oratorio choir in Helsinki for the tremendous musical experiences and good friendships as well.

Finally, I owe my deepest gratitude to my exceptionally great parents Airi and Alf for their enormous love, care, and support throughout my life.

This thesis was financially supported by grants from the Helsinki University Central Hospital Research Foundation, the Finnish Foundation for Cardiovascular Research, the Aarne Koskelo Foundation, the Finnish Medical Foundation, Finska Läkaresällskapet, Landskapsföreningen Folkhälsan på Åland, and the Wilhelm and Else Stockmann Foundation. They are all gratefully acknowledged.

Helsinki, March 2007

Marit Granér

10. REFERENCES

Abate N, Vega GL, Grundy SM. Variability in cholesterol content and physical properties of lipoproteins containing apolipoprotein B-100. *Atherosclerosis* 1993;104:159-171.

Abbott CA, Mackness MI, Kumar S, Boulton AJ, Durrington PN. Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arterioslcer Thromb Vasc Biol* 1995;15:1812-1818.

Abbott RD, Wilson PW, Kannel WB, Castelli WP. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction: the Framingham Heart Study. *Arteriosclerosis* 1988;8:207-211.

Adams MR, Nakagomi A, Keech A. Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. *Circulation* 1995;92:2127-2134.

Adkins S, Gan KN, Mody M, La Du BN. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 192, for the respective A or B allozymes. *Am J Hum Genet* 1993;52:598-608.

Agatston AS, Janowitz WR, Kaplan G, Gasso J, Hildner F, Viamonte M Jr. Ultrafast computed tomography-detected coronary calcium reflects the angiographic extent of coronary arterial atherosclerosis. *Am J Cardiol* 1994;74:1272-1274.

Aiello RJ, Brees D, Bourassa PA, Royer L, Lindsey S, Coskran T, Haghpassand M, Francone OL. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. *Arterioscler Thromb Vasc Biol* 2002;22:630-637.

Aldridge WN. Serum esterases I: two types of esterase (A and B) hydrolysing p-nitrophenyl acetate propionate and butyrate and a method for their determination. *Biochem J* 1953;53:110-117(a).

Aldridge WN. Serum esterases II: an enzyme hydrolysing diethyl p-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *Biochem J* 1953;53:117-124(b).

Alfonso F. Videodensitometric vs edge-detection quantitative angiography. Insights from intravascular ultrasound imaging. *Eur Heart J* 2000;21:604-607.

Angelin B. Therapy for lowering lipoprotein(a) levels. *Curr Opin Lipidol* 1997;8:337-341.

Arad Y, Spadaro LA, Goodman K, Newstein D, Guerci AD. Prediction of coronary events with electron beam computed tomography. *J Am Coll Cardiol* 2000;36:1253-1260.

Armstrong VW, Harrach B, Robenek H, Helmhold M, Walli AK, Seidel D. Heterogeneity of human lipoprotein lp(a): cytochemical and biochemical studies on the interaction of two lp(a) species with the LDL-receptor. *J Lipid Res* 1990;31:429-441.

Arnett EN, Isner JM, Redwood DR, Kent KM, Baker WP, Ackerstein H, Roberts WC. Coronary artery narrowing in coronary heart disease: comparison of cineangiographic and necropsy findings. *Ann Intern Med* 1979;91:350-356.

Arrol S, Mackness MI, Durrington PN. Vitamin E supplementation increases the resistance of both LDL and HDL to oxidation and increases cholesteryl ester transfer activity. *Atherosclerosis* 2000;150:129-134.

Assmann G, Schulte H. The Prospective Cardiovascular Munster (PROCAM) study: prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. *Am Heart J* 1988;116:1713-1724.

Attie AD, Kastelein JP, Hayden MR. Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis. *J Lipid Res* 2001;42:1717-1726.

Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 1988;260:1917-1921.

Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M, Erogul J, Hsu C, Dunlop C, La Du B. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arterioscler Thromb Vasc Biol* 1998;18:1617-1624(a).

Aviram M, Rosenblat M, Bisgaier CL, Newton RS. Atorvastatin and gemfibrozil metabolites, but not the parent drugs are potent antioxidants against lipoprotein oxidation. *Atherosclerosis* 1998;138:271-280(b).

Azarsiz E, Kayikcioglu M, Payzin S, Sözmen EY. PON1 activities and oxidative markers of LDL in patients with angiographically proven coronary artery disease. *Int J Cardiol* 2003;91:43-51.

Balogh Z, Fulop P, Seres I, Harangi M, Katona E, Kovacs P, Kosztaczky B, Paragh G. Effect of simvastatin on serum paraoxonase activity. *Clin Drug Invest* 2001;21:505-510.

Barter PJ, Kastelein J, Nunn A, Hobbs R. High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. *Atherosclerosis* 2003;168:195-211.

Barter PJ, Ballantyne CM, Carmena R, Castro Cabezas M, Chapman JM, Couture P, de Graaf J, Durrington PN, Faergeman O, Frohlich J, Furberg CD, Gagne C, Haffner SM, Humphries SE, Jungner I, Krauss RM, Kwiterovich P, Marcovina S, Packard CJ, Pearson TA, Reddy KS, Rosenson R, Sarrafzadegan N, Sniderman AD, Stalenhoef AF, Stein E, Talmud PJ, Tonkin AM, Walldius G, Williams KM. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. *J Intern Med* 2006;259:247-258.

Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis. Epidemiology, pathophysiology, and management. *JAMA* 2002;287:2570-2581.

Beisiegel U, Weber W, Bengtsson-Olivecrona G. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc Natl Acad Sci USA* 1991;88:8342-8346.

Berg K. A new serum type system in man – the Lp system. *Acta Pathol Microbiol Scand* 1963;59:369-382.

Bergmann K, Lutjohann D, Lindenthal B, Steinmetz A. Efficiency of intestinal cholesterol absorption in humans is not related to apoE phenotype. *J Lipid Res* 2003;44:193-197.

Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherosclerosis. *Free Radic Biol Med* 1996;20:707-727.

Bernstein MS, Costanza MC, James RW, Morris MA, Cambien F, Raoux S, Morabia A. Physical activity may modulate effects of apoE genotype on lipid profile. *Arterioscler Thromb Vasc Biol* 2002;22:133-140.

Bisgaier CL, Glickman RM. Intestinal synthesis, secretion, and transport of lipoproteins. *Annu Rev Physiol* 1983;45:625-636.

Blankenhorn DH, Selzer RH, Crawford DW, Barth JD, Liu CR, Liu CH, Mack WJ, Alaupovic P. Beneficial effects of colestipol-niacin therapy on the common carotid artery: two and four-year reduction of intima-media thickness measured by ultrasound. *Circulation* 1993;88:20-28.

Blatter MC, James RW, Messmer S, Barja F, Bometta D. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. *Eur J Biochem* 1993;211:871-879.

Blatter Garin MC, Abbott C, Messmer S, Mackness M, Durrington P, Pometta D, James RW. Quantification of human serum paraoxonase by enzyme-linked immunoassay: population differences in protein concentrations. *Biochem J* 1994;304:549-554.

Blatter Garin MC, James RW, Dussoix P, Blanche H, Passa P, Froguel P, Ruiz J. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J Clin Invest* 1997;99:62-66.

Blatter Garin MC, Kalix B, De Pree S, James RW. Aspirin use is associated with higher serum concentrations of the anti-oxidant enzyme, paraoxonase-1. *Diabetologia* 2003;46:593-594.

Boerwinkle E, Brown SA, Rohrbach K, Gotto AM, Patsch W. Role of apolipoprotein E and B gene variation in determining response of lipid, lipoprotein, and apolipoprotein levels to increased dietary cholesterol. *Am J Hum Genet* 1991;49:1145-1154.

Boman H. Cholinesterase, arylesterase and lipoproteins in twins. *Acta Genet Med Gemellol* 1980;29:281-287.

Bookstein L, Gidding SS, Donovan M, Smith FA. Day-to-day variability of serum cholesterol, triglyceride, and high-density lipoprotein cholesterol levels. Impact on the assessment of risk according to the National Cholesterol Education Program guidelines. *Arch Intern Med* 1990;150:1583-1585.

Boquist S, Ruotolo G, Tang R, Björkegren J, Bond MG, de Faire U, Karpe F, Hamsten A. Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men. *Circulation* 1999;100:723-728.

Bots ML, Evans GW, Riley WA, Grobbee DE. Carotid intima-media thickness measurements in intervention studies. Design options, progression rates, and sample size considerations: A point of view. *Stroke* 2003;34:2985-2994.

Bressler P, Bailey SR, Matsuda M, DeFronzo RA. Insulin resistance and coronary artery disease. *Diabetologia* 1996;39:1345-1350.

Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CH, Samani NJ, for the West of Scotland Coronary Prevention Study Group. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. *Lancet* 2007;369:107-114.

Brown BG, Bolson E, Frimer M, Dodge HT. Quantitative coronary arteriography. Estimation of dimensions, hemodynamic resistance, and atheroma mass of coronary artery lesions using the arteriogram and digital computation. *Circulation* 1977;55:329-337.

Brown MS, Goldstein JL. A receptor mediated pathway for cholesterol homeostasis. *Science* 1986;232:34-47.

Brunzell JD, Hazzard WR, Porte D Jr, Bierman EL. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J Clin Invest* 1973;52:1578-1585.

Budoff MJ, Achenbach S, Duerinckx A. Clinical utility of computed tomography and magnetic resonance techniques for noninvasive coronary angiography. *J Am Coll Cardiol* 2003;42:1867-1878.

Burke AP, Kolodgie FD, Farb A, Weber D, Virmani R. Morphological predictors of arterial remodeling in coronary atherosclerosis. *Circulation* 2002;105:297-303.

Caballero AE. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. *Obesity Res* 2003;11:1278-1289.

Campeau L. Grading of angina pectoris. Letter. *Circulation* 1976;54:522-523.

Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Castaldo M, Fontana L, Bonaiuto A, Bitto A, Saitta C, Saitta A. The paraoxonase promoter polymorphism (-107)T>C is not associated with carotid intima-media thickness in Sicilian hypercholesterolemic patients. *Clin Biochem* 2004;37:388-394.

Cao H, Girard-Globa A, Serusclat A, Bernard S, Bondon P, Picard S, Berthezene F, Moulin P. Lack of association between carotid intima-media thickness and paraoxonase gene polymorphism in non-insulin dependent diabetes mellitus. *Atherosclerosis* 1998;138:361-366.

Cao H, Girard-Globa A, Berthezene F, Moulin F. Paraoxonase protection of LDL against peroxidation is independent of its esterase activity towards paraoxon and is unaffected by the Q→R genetic polymorphism. *J Lipid Res* 1999;40:133-139.

Carmena R, Duriez P, Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis. *Circulation* 2004;109:III2-7.

Cashin WL, Brooks SH, Blankenhorn DH, Selzer RH, Sanmarco ME, Benjauthrit B. Computerized edge tracking and lesion measurement in coronary angiograms: a pilot study comparing smokers with non-smokers. *Atherosclerosis* 1984;52:295-300.

Cattin I, Fisicaro M, Tonizzo M, Valenti M, Danek GM, Fonda M, Da Col PG, Casagrande S, Pincetri E, Bovenzi M, Baralle F. Polymorphism of the apolipoprotein E gene and early carotid atherosclerosis defined by ultrasonography in asymptomatic adults. *Arterioscler Thromb Vasc Biol* 1997;17:91-104.

Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 2004;24:816-823.

Chapman MJ, Guerin M, Bruckert E. Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. *Eur Heart J* 1998;19:24-30.

Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CN, Kamboh MI. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the national heart lung, and blood-institute-sponsored women´s ischemia syndrome evaluation (WISE) study. *Am J Hum Genet* 2003;72:13-22(a).

Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CN, Kamboh MI. APOE polymorphism and angiographic coronary artery disease severity in the Women´s Ischemia Syndrome Evaluation (WISE) study. *Atherosclerosis* 2003;169:157-167(b).

Chen YD, Reaven GM. Intestinally-derived lipoproteins: metabolism and clinical significance. *Diabetes Metab Rev* 1991;7:191-208.

Cheng KS, Mikhailidis DP, Hamilton G, Seifalian AM. A review of the carotid and femoral intima-media thickness as an indicator of the presence of peripheral vascular disease and cardiovascular risk factors. *Cardiovasc Res* 2002;54:528-538.

Cheung MC, Albers JJ. Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. *J Lipid Res* 1982;23:747-753.

Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med* 2000;28:1815-1826.

Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treamtent: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *Lancet* 2005;366:1267-1278.

Chung BH, Segrest JP, Smith K, Griffin FM, Brouillette CG. Lipolytic surface remnants of triglyceride-rich lipoproteins are cytotoxic to macrophages but not in the presence of high-density lipoprotein: a possible mechanism to atherogenesis? *J Clin Invest* 1989;83:1363-1374.

Clarke R, Lewington S, Youngman L, Sherliker P, Peto R, Collins R. Underestimation of the importance of blood pressure and cholesterol for coronary heart disease mortality in old age. *Eur Heart J* 2002;23:286-293.

Clay MA, Newnham HH, Forte TM, Barter PI. Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apoA-I from HDL and subsequent formation of discoidal HDL. *Biochim Biophys Acta* 1992;1124:52-58.

Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Postprandial plasma lipoprotein changes in human subjects of different stages. *J Lipid Res* 1988;29:469-479.

Cohn JS, Marcoux C, Davignon J. Detection, quantification, and characterization of potentially atherogenic triglyceride-rich remnant lipoproteins. *Arterioscler Thromb Vasc Biol* 1999;19:2474-2486.

Collins P. Risk factors for cardiovascular disease and hormone therapy in women. *Heart* 2006;92(Suppl III):24-28.

Collins R, Peto R. Antihypertensive drug therapy: effects on stroke and coronary heart disease. In: Swales JD, ed. Textbook of hypertension. Oxford: Blackwell Scientific, 1992:1156-1164.

Conroy RM, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njølstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM, on behalf of the SCORE project group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003;24:987-1003.

Corella D, Tucker K, Lahoz C, Coltell O, Cupples LA, Wilson PW, Schaefer EJ, Ordovas JM. Alcohol drinking determines the effect of the APOE locus on LDL-cholesterol concentrations in men. The Framingham Offspring Study. *Am J Clin Nutr* 2001;73:736-745.

Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. *Biochem Pharm* 2005;69:541-550.

Couillard C, Bergeron N, Prud´homme D, Bergeron J, Tremblay A, Bouchard C, Mauriege P, Despres JP. Postpranidal triglyceride response in visceral obesity in men. *Diabetes* 1998;47:953-960.

Craven TE, Ryu JE, Espeland MA. Evaluation of the association between carotid artery atherosclerosis and coronary artery stenosis. *Circulation* 1990;82:1230-1242.

Criqui MH, Heiss G, Cohn R, Cowan LD, Suchindran CM, Bangdiwala S. Plasma triglyceride level and mortality from coronary heart disease. *N Engl J Med* 1993;328:1220-1225.

Crouse JR, Craven TE, Hagaman AP, Bond MG. Association of coronary disease with segment-specific intimal-medial thickening of extracranial carotid artery. *Circulation* 1995;92:1141-1147.

Crouse JR, Goldbourt U, Evans G, Pinsky J, Sharret AR, Sorlie P, Riley W, Heiss G. Risk factors and segment-specific carotid arterial enlargement in the Atherosclerosis Risk in communities (AIRC) cohort. *Stroke* 1996;27:69-75.

Crouse JR 3rd. Predictive value of carotid 2-dimensional ultrasound. *Am J Cardiol* 2001;88:27E-30E.

Cruickshank JM, Neil-Dwyer G, Dorrance DE, Hayes Y, Patel S. Acute effects of smoking on blood pressure and cerebral blood flow. *J Hum Hypertens* 1989;3:443-449.

Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. *Am J Cardiol* 2000;86:943-949.

Curtiss LK, Boisvert WA. Apolipoprotein E and atherosclerosis. *Curr Opin Lipidol* 2000; 11:243-251.

Cutler JA, Psaty BM, MacMahon S, Furberg CD. Public health issues in hypertension control: what has been learned from clinical trials. In Laragh JH, Brenner BM, eds. Hypertension: Pathopshyiology, diagnosis, and management. 2nd ed. New York City, NY: Raven Press Publishers; 1995:253-270.

Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype. *J Lipid Res* 1992;33:447-454.

Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. *Circulation* 2000;102:1082-1085.

Davidson WS, Sparks DL, Lund-Katz S, Phillips MC. The molecular basis for the difference in charge between pre-beta- and alpha-migrating high density lipoproteins. *J Biol Chem* 1994;269:8959-8965.

Davignon J, Cohn JS, Mabile L, Bernier L. Apolipoprotein E and atherosclerosis: insight from animal and human studies. *Clin Chim Acta* 1999;286:115-143.

Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age. *Circulation* 2001;104:2815-2819.

Deakin S, Moren X, James RW. Very low density lipoproteins provide a vector for secretion of paraoxonase-1 from cells. *Atherosclerosis* 2005;179:17-25.

De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, Ebrahim S, Faergeman O, Graham I, Mancia G, Cats VM, Orth-Gomer K, Perk J, Pyörälä K, Rodicio JL, Sans S, Sansoy V, Secthem U, Silber S, Thomsen T, Wood D. European guidelines on cardiovascular disease prevention in clinical practice. *Eur J Cardiovasc Prev Rehabil* 2003;10:S1-78.

de Feyter PJ, Serruys PW, Davies Mj, Richardson P, Lubsen J, Oliver MF. Quantitative coronary angiography to measure progression and regression of coronary atherosclerosis. Value, limitations, and implications for clinical trials. *Circulation* 1991;84:412-423.

De Franco A, Nissen SE. Coronary intravascular ultrasound: implications for understanding the development and potential regression of atherosclerosis. *Am J Cardiol* 2001;88:7M-20M.

DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991;14:173-194.

de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healty subjects. *Arterioscler Thromb* 1991;11:298-306.

Demant T, Carlson LA, Holmqvist L, Karpe F, Nilsson-Ehle P, Packard CJ, Shepherd J. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J Lipid Res* 1988;29:1603-1611.

Denborough MA. Alimentary lipemia in ischemic heart disease. *Clin Sci* 1963;25:115-122.

Després JP, Lamarche B, Mauriége P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Eng J Med* 1996;334:952-957.

Dessi M, Gnasso A, Motti C, Pujia A, Irace C, Casciani S, Staffa F, Federici G, Cortese C. Influence of the human paraoxonase polymorphism (PON1 192) on the carotid-wall thickening in a healthy population. *Coron Artery Dis* 1999;10:595-599.

Devaraj S, Vega G, Lange R, Grundy SM, Jialal I. Remnant-like particle cholesterol levels in patients with dysbetalipoproteinemia or coronary artery disease. *Am J Med* 1998;104:445-450.

Devlin CM, Lee SJ, Kuriakose G, Spencer C, Becker L, Grosskopf I, Ko C, Huang LS, Koschinsky ML, Cooper AD, Tabas I. An apolipoprotein(a) peptide delays chylomicron remnant clearance and increases plasma remnant lipoproteins and atherosclerosis in vivo. *Arterioscler Thromb Vasc Biol* 2005;25:1704-1710.

Diwadkar VA, Anderson JW, Bridges SR, Gowri MS, Oelgten PR. Postprandial low-density lipoproteins in type 2 diabetes are oxidized more extensively than fasting diabetes and control samples. *Proc Soc Exp Biol Med* 1999;222:178-184.

Durrington PN, Hunt L, Ishola J, Kane J, Stephens WP. Serum apolipoproteins A-I and B and lipoproteins in middle-aged men with and without previous myocardial infarction. *Br Med J* 1986;56:206-212.

Durrington PN. Lipoprotein (a). *Baillieres Clin Endocrinol Metab* 1995;9:773-795.

Durrington PN, Mackness MI, Bhatnagar D, Julier K, Prais H, Arrol S, Morgan J, Wood GN. Effects of two different fibric acid derivatives on lipoproteins, cholesteryl ester transfer, fibrinogen, plasminogen activator inhibitor and paraoxonase activity in type IIb hyperlipoproteinaemia. *Atherosclerosis* 1998;138:217-225.

Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001;21:473-480.

Eckerson HW, Romson J, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. *Am J Hum Genet* 1983;35:214-227(a).

Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet* 1983;35:1126-1138(b).

Eichner JE, Kuller LH, Orchard TJ, Grandits GA, McCallum LM, Ferrell RE, Neaton JD. Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. *Am J Cardiol* 1993;71:160-165.

Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *Am J Epidemiol* 2002;155:487-495.

Eisenberg S. High density lipoprotein metabolism. *J Lipid Res* 1984;25:1017-1058.

Elosua R, Ordovas JM, Cupples LA, Fox CS, Polak JF, Wolf PA, D'Agostino RA Sr, O'Donnel CJ. Association of APOE genotype with carotid atherosclerosis in men and women: the Framingham Heart Study. *J Lipid Res* 2004;45:1868-1875.

Enas EA, Chacko V, Senthilkumar A, Puthumana N, Mohan V. Elevated lipoprotein(a) – a genetic risk factor for premature vascular disease in people with and without standard risk factors: a review. *Dis Mon* 2006;52:5-50.

Erdös EG, Boggs LE. Hydrolysis of paraxon in mammalian blood. *Nature* 1961;190:716-717.

Espeland MA, Tang R, Terry JG, Davis DH, Mercuri M, Crouse JR. Associations of risk factors with segment-specific intimal-medial thickness of the extracranial carotid artery. *Stroke* 1999;30:1047-1055.

Espeland MA, O´Leary DH, Terry JG, Morgan T, Evans G, Mudra H. Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. *Curr Control Trials Cardiovasc Med* 2005;10:6(1):3.

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143-3421.

Falk E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol* 2006;47:C7-12.

Faxon DP, Fuster V, Libby P, Beckman JA, Hiatt WR, Thompson RW, Topper JN, Annex BH, Rundback JH, Fabunmi RP, Robertson RM, Loscalzo J. Atherosclerotic vascular disease conference: Writing Group III: pathophysiology. *Circulation* 2004;109:2617-2625.

Fernández-Miranda C, Aranda JL, Martin MA, Arenas J, Núñez V, de la Cámara G. Apolipoprotein E polymorphism and carotid atherosclerosis in patients with coronary artery disease. *Int J Cardiol* 2004;94:209-212.

Ferrara A, Barrett-Connor EL, Edelstein SL. Hyperinsulinemia does not increase the risk of fatal cardiovascular disease in elderly men or women without diabetes: the Rancho Bernardo Study, 1984-1991. *Am J Epidemiol* 1994;140:857-869.

Fischer M, Broeckel U, Holmer S, Baessler A, Hengstenberg C, Mayer B, Erdmann J, Klein G, Riegger G, Jacob HJ, Schunkert H. Distinct heritable patterns of angiographic coronary artery disease in families with myocardial infarction. *Circulation* 2005;111:855-862.

Fleming RM, Kirkeeide RL, Smalling RW, Gouild KL. Patterns in visual interpretation of coronary angiograms as detected by quantitative coronary angiography. *J Am Coll Cardiol* 1991;18:945-951.

Flugel M, Geldmacher-von Mallinckrodt M. On kinetics of the paraoxon hydrolysing enzyme in human serum (EC 3.1.1.2) (author´s transl). Klin Wochenschr 1978;56:911-916.

Fruchart JC, Nierman MC, Stroes E, Kastelein J, Duriez P. New risk factors for atherosclerosis and patient risk assessment. *Circulation* 2004;109:15-19.

Fukushima H, Kugiyama K, Sugiyama S, Honda O, Koide S, Nakamura S, Kawano H, Soejima H, Miyamato S, Yoshimura M, Sakamoto T, Ogawa H. Comparison of remnant-like lipoprotein particles in postmenopausal women with and without coronary artery disease and in men with coronary artery disease. *Am J Cardiol* 2001;88:1370-1373.

Furlong CE, Costa LG, Hassett C, Richter RJ, Sundstrom JA, Adler DA, Disteche CM, Omiecinski CJ, Chapline C, Crabb JW, Humbert R. Human and rabbit paraoxonase: purification, cloning, sequencing, mapping and role of polymorphism in organophosphate detoxification. *Chem Biol Interact* 1993;87:35-48.

Gariepy J, Salomon J, Denarie N, Laskri F, Megnien JL, Levenson J, Simon A. Sex and topographic differences in associations between large-artery wall thickness and coronary risk profile in a French working cohort: the AXA study. *Arterioscler Thromb Vasc Biol* 1998;18:584-590.

Gensini GG. A more meaningful scoring system for determining the severity of coronary artery disease. *Am J Cardiol* 1983;51:606.

Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N Engl J Med* 1994;330:1431-1438.

Ginsberg HN. Lipoprotein physiology and its relationship to atherogenesis. *Endocrinol Metab Clin North Am* 1990;19:211-228.

Glagov SG, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med* 1987;316:1371-1375.

Glagov SG, Zarins C, Giddens DP, Ku DN. Hemodynamics and atherosclerosis. *Arch Patol Lab Med* 1988;112:1018-1031.

Goldberg RK, Kleiman NS, Minor ST, Abukhalil J, Raizner AE. Comparison of quantitative coronary angiography to visual estimates of lesion severity pre and post PTCA. *Am Heart J* 1990;119:178-184.

Goldstein JL, Ho YK, Basu SK, Brown MS. Binding sites on macrophages that mediate uptake and degradation of acetylated low-density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci* 1979;76:333-337.

Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR Jr, Bangdiwala S, Tyroler A. High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. *Circulation* 1989;79:8-15.

Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High-density lipoprotein as a protective factor against coronary heart disease: the Framingham Study. *Am J Med* 1977;62:707-714.

Gotto AM Jr, Pownall HJ, Havel RJ. Introduction to the plasma lipoproteins. *Methods Enzymol* 1986;128:3-41.

Gotto AM, Pownall H eds. Atherosclerosis: Overview and histologic classification of lesions. In: Manual of lipid disorders. Reducing the risk of coronary heart disease. Baltimore: Williams & Wilkins, 1999:56-66.

Grebe MT, Schoene E, Schaefer CA, Boedeker RH, Kemkes-Matthes B, Voss R, Tillmanns HH. Elevated lipoprotein(a) does not promote early atherosclerotic changes of the carotid arteries in young, healthy adults. *Atherosclerosis* 2007;190:194-198.

Green PH, Glickman RM. Intestinal lipoprotein metabolism. *J Lipid Res* 1981;22:1153-1173.

Greenland P, Sidney SC, Grundy SM. Improving coronary heart disease risk assessment in asymptomatic people. Role of traditional risk factors and noninvasive cardiovascular tests. *Circulation* 2001;104:1863-1867.

Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. *Atherosclerosis* 1990;83:59-67.

Griffin M, Nicolaides AN, Belcaro G, Shah E. Cardiovascular risk assessment using ultrasound: the value of arterial wall changes including the presence, severity and character of plaques. *Pathophysiol Haemost Thromb* 2002;32:367-370.

Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arterioscler Thromb* 1991;11:653-662.

Grover SA, Coupal L, Hu X-P. Identifying adults at increased risk of coronary disease. How well do the current cholesterol guidelines work? *JAMA* 1995;274:801-806.

Grundy SM, Balady GJ, Criqui MH, Fletcher G, Greenland P, Hiratzka LF, Houston-Miller N, Kris-Etherton P, Krumholz HM, LaRosa J, Ockene IS, Pearson TA, Reed J, Washington R, Smith SC Jr. Primary prevention of coronary heart disease: guidance from Framingham. A statement for healthcare professionals from the AHA task force on risk reduction. *Circulation* 1998;97:1876-1887.

Grundy SM, Pasternak R, Greenland P, Smith S Jr, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations. A statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *Circulation* 1999;100:1481-1492.

Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;339:229-234.

Hanley AJ, Williams K, Stern MP, Haffner SM. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease. *Diabetes Care* 2002;25:1177-1184.

Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685-1695.

Hartung GH, Lawrence SJ, Reeves RS, Foyet JP. Effect of alcohol in exercise on post-prandial lipemia and triglyceride clearance in men. *Atherosclerosis* 1993;100:33-40.

Hasselwander O, McMaster D, Fogarty DG, Maxwell AP, Nicholls DP, Young IS. Serum paraoxonase and platelet-activating factor acetylhydrolase in chronic renal failure. *Clin Chem* 1998;44:179-181.

Havekes LM, de Knijff P, Beisiegel U, Havinga J, Smit M, Klasen E. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J Lipid Res* 1987;28:455-463.

Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345-1353.

Havel RJ. Role of triglyceride-rich lipoproteins in progression of atherosclerosis. *Circulation* 1990;81:694-696.

Havel RJ. Postprandial hyperlipidemia and remnant lipoprotein. *Curr Opin Lipidol* 1994;5:102-109.

Heeren J, Beisiegel U. Intracellular metabolism of triglyceride-rich lipoproteins. *Curr Opin Lipidol* 2001;12:255-260.

Heeren J, Grewal T, Laatsch A, Becker N, Rinninger F, Rye KA, Beisiegel U. Impaired recycling of apolipoprotein E4 is associated with intracellular cholesterol accumulation. *J Biol Chem* 2004;279:55483-55492.

Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the Aric study. *Am J Epidemiol* 1991;134:250-256.

Henry P, Makowski S, Richard P, Beverelli F, Casanova S, Louali A, Boughalem K, Battaglia S, Guize L, Guermonprez JL. Increased incidence of moderate stenosis among patients with diabetes: substrate for myocardial infarction? *Am Heart J* 1997;134:1037-1043.

Herrington DM, Bean JA, Mercuri M. Strong correlation between carotid artery wall thickness and quantitative coronary angiographic assessment of coronary atherosclerosis. Abstract. *Circulation* 1994;90:I-534.

Holaj R, Spacil J, Petrasek J, Malik J, Haas T, Aschermann M. Intima-media thickness of the common carotid artery is the significant predictor of angiographically proven coronary artery disease. *Can J Cardiol* 2003;19:670-676.

Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 1998;98:1487-1494.

Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E, Van de Werf F. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2001;21:844-848.

Holvoet P, Kritchevsky SB, Tracy RP, Mertens A, Rubin SM, Butler J, Goodpaster B, Harris TB. The metabolic syndrome, circulating oxidized LDL, and risk of myocardial infarction in well-functioning elderly people in the Health, Aging, and Body Composition Cohort. *Diabetes* 2004;4:1068-1073.

Hong S, Zhao S, Wu Z. Probucol up-regulates paraoxonase 1 expression in hepatocytes of hypercholesterolemic rabbits. *J Cardiovasc Pharmacol* 2006;47:77-81.

Hu FB, Sampfer MJ, Solomon CG, Liu S, Willett W, Speizer FE, Nathan DM, Manson JE. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. *Arch Intern Med* 2001;161:1717-1723.

Hubert HB, Holford TR, Kannel WB. Clinical characteristics and cigarette smoking in relation to prognosis of angina pectoris in Framingham. *Am J Epidemiol* 1982;115:231-242.

Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 1998;280:605–613.

Hulley SB, Rosenman RH, Bawol RD, Brand RJ. Epidemiology as a guide to clinical decisions: the association between triglyceride and coronary heart disease. *N Engl J Med* 1980;302:1383-1389.

Hulthe J, Bokemark L, Wikstrand J, Fagerberg B. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (ARIC) study. *Arterioscler Thromb Vasc Biol* 2000;20:2140-2147(a).

Hulthe J, Wiklund O, Bondjers G, Wikstrand J. LDL particle size in relation to intima-media thickness and plaque occurrence in the carotid and femoral arteries in patients with hypercholesterolaemia. *J Int Med* 2000;248:42-52(b).

Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arterioscler Thromb Vasc Biol* 2002;22:1162-1167.

Humphrey LL, Chan B, Sox HS. Postmenopausal hormone replacement therapy and the primary prevention of cardiovascular disease. *Ann Intern Med* 2002;137:273-284.

Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN, Miller GJ. Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *Lancet* 2001;358:115-119.

Hutchins GM, Bulkley BH, Ridolfi RL, Griffith LS, Lohr FT, Piasio MA. Correlation of coronary arteriograms and left ventriculograms with postmortem studies. *Circulation* 1977;56:32-37.

Imke C, Rodriquez BL, Grove JS, McNamara JR, Waslien C, Katz AR, Willcox B, Yano K, Curb D. Are remnant-like particles independent predictors of coronary heart disease incidence? The Honolulu Heart Study. *Arterioscler Thromb Vasc Biol* 2005;25:1718-1722.

Ishizaka N, Ishizaka Y, Takahashi E, Unuma T, Tooda E, Nagai R, Togo M, Tsukamoto K, Hashimoto H, Yamakado M. Association between insulin resistance and carotid arteriosclerosis in subjects with normal fasting glucose and normal glucose tolerance. *Arterioscler Thromb Vasc Biol* 2003;23:295-301.

Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001;24:683-689.

Itabe H, Yamamoto H, Imanaka T, Shimamura K, Uchiyama H, Kimura J, Sanaka T, Hata Y, Takano T. Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. *J Lipid Res* 1996;37:45-53.

James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in coronary artery disease patients. *Circulation* 2000;101:2252-2257.

Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, Furlong CE. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is $PON1_{192}$ or $PON1_{55}$ genotype. *Arterioscler Thromb Vasc Biol* 2000;20:2441-2447.

Jarvik GP, Tsai NT, McKinstry LA, Wani R, Brophy VH, Richter RJ, Schellenberg GD, Heagerty PJ, Hatsukami TS, Furlong CE. Vitamin C and E intake is associated with increased paraoxonase activtiy. *Arterioscler Thromb Vasc Biol* 2002;22:1329-1333.

Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL. Biological variation of homeostasis model assessment-derived insulin resistance in type 2 diabetes. *Diabetes Care* 2002;25:2022-2025.

Juutilainen A, Kortelainen S, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Gender difference in the impact of type 2 diabetes on coronary heart disease risk. *Diabetes Care* 2004;27:2898-2904.

Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005;26:439-451.

Kannel WB, Gordon T. Evaluation of cardiovascular risk in the elderly: the Framingham Study. *Bull N Y Acad Med* 1978;54:573-591.

Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. *JAMA* 1979;241:2035-2038.

Kannel WB, Wilson PW. Risk factors that attenuate the female coronary disease advantage. *Arch Intern Med* 1995;155:57-61.

Kannel WB. Blood pressure as a cardiovascular risk factor: prevention and treatment. *JAMA* 1996;275:1571-1576.

Kanters SD, Algra A, van Leeuwen MS, Banga JD. Reproducibility of in vivo carotid intima-media thickness measurements: a review. *Stroke* 1997;28:665-671.

Kaplan M, Hayek T, Raz A, Coleman R, Dornfeld L, Vaya J, Aviram M. Pomegrante juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. *J Nutr* 2001;131:2082-2089.

Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 1994;106:83-97.

Karpe F, de Faire U, Mercuri M, Bond MG, Hellénius M-L, Hamsten A. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. *Atherosclerosis* 1998;141:307-314.

Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. *J Intern Med* 1999;246:341-355.

Karpe F, Hellénius M-L, Hamsten. Differences in postprandial concentrations of very-low-density lipoprotein and chylomicron remnants between normotriglyceridemic and hypertriglyceridemic men with and without coronary heart disease. *Metabolism* 1999;48:301-307.

Karpe F, Boquist S, Tang R, Bond GM, de Faire U, Hamsten A. Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. *J Lipid Res* 2001;42:17-21.

Kawachi I, Colditz GA, Stampfer MJ, Wilett WC, Manson JE, Rosner B, Speizer FE, Hennekens CH. Smoking cessation in relation to total mortality rates in women. A prospective cohort study. *Ann Intern Med* 1993;119:992-1000.

Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;89:2548-2556.

Koba S, Tsunoda F, Hirano T, Iso Y, Suzuki H, Geshi E, Katagiri T. Postprandial changes in LDL phenotypes in patients with myocardial infarction. *Eur J Clin Invest* 2005;35:171-179.

Kolovou G, Daskalova D, Mikhailidis DP. Apolipoprotein E polymorphism and atherosclerosis. *Angiology* 2003;54:59-71.

Kool MJ, Hoeks AP, Struijker Boudier HA, Reneman RS, Van Bortel LM. Short- and long-term effects of smoking on arterial wall properties in habitual smokers. *J Am Coll Cardiol* 1993;22:1881-1886.

Kopprash S, Pietzsch J, Kulisch E, Fuecker K, Temelkova-Kurktschiev T, Hanefeld M, Kuhne H, Julius U, Graessler J. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. *Diabetes* 2002;51:3102-3106.

Kornowski R, Mintz GS, Lansky AJ, Hong MK, Kent KM, Pichard AD, Satler LF, Popma JJ, Bucher TA, Leon MB. Paradoxic decreases in atherosclerotic palque mass in insulin-treated diabetic patients. *Am J Cardiol* 1998;81:1298-1304.

Korpilahti K, Syvänne M, Engblom E, Hämäläinen H, Puukka P, Rönnemaa T. Components of the insulin resistance syndrome are associated with progression of atherosclerosis in non-grafted arteries 5 years later after coronary artery bypass surgery. *Eur Heart J* 1998;19:711-719.

Kostner GM. Interaction of lp(a) and of apo(a) with liver cells. *Arterioscler Thromb* 1993;13:1101-1109.

Krasinski SD, Cohn JS, Schaefer EJ, Russell RM. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. *J Clin Invest* 1990;85:883-892.

Krauss RM, Blanche PJ. Detection and quantification of LDL subfractions. *Curr Opin Lipidol* 1992;3:377-383.

Krauss RM. Atherogenicity of triglyceride-rich lipoproteins. *Am J Cardiol* 1998;81:13B-17B.

Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells in vivo: a specific vascular action of insulin. *Circulation* 2000;101:678-681.

Kugiyama M, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, Tsunoda R, Sakamoto T, Nakano T, Nakajima K, Ogawa H, Sugiyama S, Yoshimura M, Yasue H. Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation* 1999;99:2858-2860.

Kujiraoka T, Oka T, Ishihara M, Egashira T, Fujioka T, Saito E, Saito S, Miller NE, Hattori H. A sandwich enzyme-linked immunosorbent assay for human serum paraoxonase concentration. *J Lipid Res* 2000;41:1358-1363.

Lada A, Rudel LL. Associations of low-density lipoprotein particle composition with atherogenicity. *Curr Opin Lipidol* 2004;15:19-24.

La Du BN, Novais J. Human serum organophosphatase: biochemical characteristics and polymorphic inheritance. In Enzymes Hydrolysing Organophosphorus Compounds 1989; pp. 41-52.

La Du BN. Future studies of low-activity PON1 phenotype subjects may reveal how PON1 protects against cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2003;23:1317-1318.

Lakka TA, Salonen R, Kaplan GA, Salonen JT. Blood pressure and the progression of carotid atherosclerosis in middle-aged men. *Hypertension* 1999;34:51-56.

Lamarche B, Moorjani S, Lupien PJ, Cantin B, Bernard PM, Dagenais GR, Despres JP. Apolipoprotein A-I and B levels and the risk of ischemic heart study disease during a five-year follow-up of men in the Québec Cardovascular Study. *Circulation* 1996;94:273-278.

Lawn RM, Wade DP, Garvin MR, Wang X, Schwartz K, Porter JG, Seilhamer JJ, Vaughan AM, Oram JF. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J Clin Invest* 1999;104:R25-31.

Lefevre M, Ginsberg HN, Kris-Etherton PM, Elmer PJ, Stewart PW, Ershow A, Pearson TA, Roheim PS, Ramakrishnan R, Derr J, Gordon DJ, Reed R. ApoE genotype does not predict lipid response to changes in dietary saturated fatty acids in a heterogeneous normolipidemic population. The DELTA Research Group. Dietary Effects on Lipoproteins and Thrombogenic Activity. *Arterioscler Thromb Vasc Biol* 1997;17:2914-2923.

Lehtimäki T, Lehtinen S, Solakivi T, Nikkilä M, Jaakkola O, Jokela H, Ylä-Herttuala S, Luoma JS, Koivula T, Nikkari T. Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *Arterioscler Thromb Vasc Biol* 1999;19:23-27.

Lekakis JP, Papamichael CM, Cimponeriu AT, Stamatelopoulos KS, Papaioannou TG, Kanakakis J, Alevizaki MK, Papapanagiotou A, Kalofoutis AT, Stamatelopoulos SF. Atherosclerotic changes of extra-coronary arteries are asscociated with the extent of coronary atherosclerosis. *Am J Cardiol* 2000;85:949-952.

Lewis GF, O´Meara NM, Soltys PA, Blackman JD, Iverius PH, Druetzler AF, Getz GS, Polonsky KS. Postprandial lipoprotein metabolism in normal and obese subjects: comparison after the vitamin A fat-loading test. *J Clin Endocrinol Metab* 1990;71:1041-1050.

Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 1993;329:1988-1992.

Lin CY, Duan HW, Mazzone T. Apolipoprotein E-dependent cholesterol efflux from macrophages: kinetic study and divergent mechanism for endogenous apolipoprotein E. *J Lipid Res* 1999;40:1618-1626.

Lindenstrom E, Boysen G, Nyboe J. Influence of total cholesterol, high density lipoprotein cholesterol, and triglycerids on risk of cerebrovascular disease: the Copenhagen City Heart Study. *BMJ* 1994;309:11-15.

Lindgren FT, Jensen LC, Wills RD, Freeman NK. Flotation rates, molecular weights and hydrated densities of low-density lipoproteins. *Lipids* 1969;4:334-337.

Liu S, Ma J, Ridker PM, Breslow JL, Stampfer MJ. A prospective study of the association between APOE genotype and the risk of myocardial infarction among apparently healthy men. *Atherosclerosis* 2003;166:323-329.

Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness. *Circulation* 2007;115:459-467.

Lotufo RA, Gaziano J, Michael MD, Chae CU, Ajani UA, Moreno-John G, Buring J, Manson JE. Diabetes and all-cause and coronary heart disease mortality among US male physicians. *Arch Intern Med* 2001;161:242-247.

Luc G, Bard JM, Arveiler D, Ferrieres J, Evans A, Amouyel P, Fruchart JC, Ducimetiere P. Lipoprotein (a) as a predictor of coronary heart disease: the PRIME study. *Atherosclerosis* 2002;163:377-384.

Mack WJ, Selzer RH, Pogoda JM, Lee PL, Shircore AM, Azen SP, Blankenhorn DH. Comparison of computer- and human-derived coronary angiographic end-point measures for controlled therapy trials. *Arterioscler Thromb* 1992;12:348-356.

Mack WJ, LaBree L, Liu CR, Liu CH, Selzer RH, Hodis HN. Correlations between measures of atherosclerosis change using carotid ultrasonography and coronary angiography. *Atherosclerosis* 2000;150:371-379.

Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, Roberts C, Durrington PN, Mackness MI. Paraoxonase status in coronary heart disease. Are activity and concentration more important than genotype? *Arterioscler Thromb Vasc Biol* 2001;21:1451-1457.

Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, Mackness M. Low paraoxonase activity predicts coronary events in the Caerphilly prospective study. *Circulation* 2003;107:2775-2779.

Mackness MI, Thompson HM, Hardy AR, Walker CH. Distinction between ´A´-esterases and arylesterases. Implications for esterase classification. *Biochem J* 1987;245:293-296.

Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis* 1991;86:193-198.

Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis* 1993;104:129-135.

Mackness MI, Mackness B, Durrington PN. Paraoxonase and coronary heart disease. *Atherosclerosis* 2002;3:49-55.

MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 1990;335:765-774.

Mahley RW, Rall SC Jr. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In The Metabolic and Molecular Bases of Inherited Disease. 7th edition. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, editors. McGraw-Hill, New York, 1995:1953-1980.

Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;1:507-537.

Manning WJ, Li W, Edelman RR. A preliminary report comparing magnetic resonance coronary angiography with conventional angiography. *N Engl J Med* 1993;328:828-832.

Marais AD. Therapeutic modulation of low-density lipoprotein size. *Curr Opin Lipidol* 2000;11:597-602.

Marcoux C, Tremblay M, Jacques H, Krimbou L, Fredenrich A, Nakajima K, Davignon J, Cohn JS. Plasma remnant-like particle lipid and apolipoprotein levels in normolipidemic and hyperlipidemic subjects. *Atherosclerosis* 1998;139:161-171.

Marcovina SM, Albers JJ, Kennedy H, Mei JV, Henderson O, Hannon WH. International Federation of Clinical Chemistry standardization project for measurement of apolipoproteins A-I and B. IV, comparability of apolipoprotein B values by use of international reference material. *Clin Chem* 1994;40:586-592.

Marcovina SM, Koschinsky ML. Lipoprotein(a) as a risk factor for coronary artery disease. *Am J Cardiol* 1998;83:57U-66U.

Marcus ML, Skorton DJ, Johnson MR, Collins SM, Harrison DG, Kerber RE. Visual estimates of percent diameter coronary stenosis: "a battered gold standard". *J Am Coll Cardiol* 1988;11:882-885.

Markus H, Kapozsta Z, Ditrich R, Wolfe C, Ali N, Powell J, Mendell M, Cullinane M. Increased common carotid intima-media thickness in UK African Caribbeans and its relation to chronic inflammation and vascular candidate gene polymorphisms. *Stroke* 2001;32:2465-2471.

Masuoka H, Kamei S, Ozaki M, Kawasaki A, Shintani U, Ito M, Nakano T. Predicive value of remnant-like particle cholesterol as an indicator of coronary artery stenosis in patients with normal serum triglyceride levels. *Intern Med* 2000;39:540-546.

Matthews D, Lang D, Burnett M, Turner R. Control of pulsatile insulin secretion in man. *Diabetologia* 1983;24:231-237.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RA. Homeostasis model assessment: insulin resistance and β cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.

Mautner GC, Mautner SL, Froehlich J, Feurstein IM, Proschan MA, Roberts WC, Doppman JL. Coronary artery calcification: assessment with electron beam CT and histomorphometric correlation. *Radiology* 1994;192:619-623.

Mayr M, Kiechl S, Tsimikas S, Miller E, Sheldon J, Willeit J, Witztum JL, Qingbo X. Oxidized low-density lipoprotein antibodies, chronic infections, and carotid atherosclerosis in a population-based study. *J Am Coll Cardiol* 2006;47:2436-2443.

Mazurek T, Zhang L, Zalewski A, Mannon JD, Diehl JT, Arafat H, Sarov-Blat L, Obrien S, Keiper E, Johnson AG, Martin J, Goldstein BJ, Shi Y. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation* 2003;108:2460-2466.

McNamara JR, Shah PK, Nakajima K, Cupples LA, Wilson PW, Ordovas JM Schaefer EJ. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. *Atherosclerosis* 2001;154:229-236.

Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation* 2005;112:651-657.

Mekki N, Christofilis MA, Charbonnier M, Atlan-Gepner C, Defoort C, Juhel C, Borel P, Portugal H, Pauli AM, Vialettes B, Lairon D. Influence of obesity and body fat distribution on postprandial lipemia and triglyceride-rich lipoproteins in adult women. *J Clin Endocrinol Metab* 1999;84:184-191.

Mero N, Malmström R, Steiner G, Taskinen MR, Syvänne M. Postprandial metabolism of apolipoprotein B-48 and B-100 containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. *Atherosclerosis* 2000;150:167-177.

Merril JR, Holly RG, Anderson RL, Rifai N, King ME, DeMeersman R. Hyperlipemic response of young trained and untrained men after a high fat meal. *Arteriosclerosis* 1989;9:217-223.

Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J* 2001;15:2073-2084.

Miettinen H, Lehto S, Salomaa V, Mähönen M, Niemelä M, Haffner S, Pyöralä K, Tuomilehto J. Impact of diabetes on mortality after the first myocardial infarction. *Diabetes Care* 1998;21:69-75.

Miettinen TA, Gylling H, Vanhanen H, Ollus A. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arterioscler Thromb* 1992;12:1044-1052.

Millar JS, Lichtenstein AH, Cuchel M, Dolnikowski GG, Hachey DL, Cohn JS, Schaefer EJ. Impact of age on the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 in men. *J Lipid Res* 1995;36:1155-1167.

Miller NE, Thelle DS, Førde OH, Mjøs OD. The Tromsø heart-study: high-density lipoprotein and coronary heart disease: a prospective case-control study. *Lancet* 1977;1:965-968.

Moise A, Clement B, Saltiel J. Clinical and angiographic correlates and prognostic significance of the coronary extent score. *Am J Cardiol* 1988;61:1255-1259.

Moliterno DJ, Jokinen EV, Miserez AR, Lange RA, Willard JE, Boerwinkle E, Hillis LD, Hobbs HH. No association between plasma lipoprotein(a) concentrations and the presence or absence of coronary atherosclerosis in African Americans. *Arterioscler Thromb Vasc Biol* 1995;15:850-855.

Moser M, Hebert P, Hennekens CH. An overview of the meta-analyses of the hypertension treatment trials. *Arch Intern Med* 1991;151:1277-1279.

Moss AJ, Goldstein RE, Marder VJ, Sparks CE, Oakes D, Greenberg H, Weiss HJ, Zareba W, Brown MW, Liang CS, Lichstein E, Little WC, Gillespie JA, Van Voorhees L, Krone RJ, Bodenheimer MM, Hochman J, Dwyer EM Jr, Arora R, Marcus FI, Watelet LF, Case RB. Thrombogenic factors and recurrent coronary events. *Circulation* 1999;99:2517-2522.

Mounter LA, Whittaker VP. The hydrolysis of esters of phenol by cholinesterases and other esterases. *Biochem J* 1953;54:551-559.

Mueller RF, Hornung S, Furlong CE, Anderson J, Giblett ER, Motulsky AG. Plasma paraxonase polymorphism: a new enzyme assay, population, family, biochemical and linkage studies. *Am J Hum Genet* 1983;35:393-408.

Myerburg RJ, Kessler KM, Castellanos A. Sudden cardiac death: epidemiology, transient risk, and intervention assessment. *Ann Intern Med* 1993;119:1187-1197.

Mykkänen L, Kuusisto J, Haffner SM, Laakso M, Austin MA. LDL size and risk of coronary heart disease in elderly men and women. *Arterioscler Thromb Vasc Biol* 1999;19:2742-2748.

März W, Beckmann A, Scharnagl H, Siekmeier R, Mondorf U, Held I, Schneider W, Preissner KT, Curtiss LK, Gross W, Huttinger M. Heterogeneous lipoprotein (a) size isoforms differ by their interaction with the low density lipoprotein receptor and the low density lipoprotein receptor-related protein/α2-macroglobulin receptor. *FEBS* 1993;325:271-275.

Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E. Cholesterol in remnant like lipoproteins in human serum using monoclonal anti apoB-100 and apoA-I immunoaffinity mixed gel. *Clin Chim Acta* 1993;223:53-71.

Nakamura Y, Shimada K, Fukuda D, Shimada Y, Ehara S, Hirose M, Kataoka T, Kamimori K, Shimodozomo S, Kobayashi Y, Yoshiyama M, Takeuchi K, Yoshikawa J. Implications of plasma concentrations of adiponectin in patients with coronary artery disease. *Heart* 2004;90:528-533.

Neaton JD, Blackburn H, Jacobs D, Kuller L, Lee DJ, Sherwin R, Shih J, Stamler J, Wentworth D. Serum cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. *Arch Intern Med* 1992;152:1490-1500.

Nestel PJ. Relationship between plasma triglycerides and removal of chylomicrons. *J Clin Invest* 1964;43:943-949.

Ng CJ, Shih DM, Hama SY, Villa N, Navab M, Srinivasa TR. The paraoxonase gene family and atherosclerosis. *Free Radic Biol Med* 2005;38:153-163.

Nichols AV, Krauss RM, Musliner TA. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol* 1986;128:417-431.

Nikkilä E. Studies on the lipid-protein relationships in normal and pathologic sera and the effect of heparin on serum lipoproteins. *Scand J Clin Lab Invest* 1953;5:9-101.

Nikkilä EA, Konttinen A. Effect of physical activity on postprandial levels of fats in serum. *Lancet* 1962;1:1151-1154.

Nishioka T, Nagai T, Luo H, Kitamura K, Hakamata N, Akanuma M, Katsushika S, Uehata A, Takase B, Isojima K, Ohtomi S, Siegel RJ. Coronary remodeling of proximal and distal stenotic atherosclerotic plaques within the same artery by intravascular ultrasound study. *Am J Cardiol* 2001;87:387-391.

Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* 2002;161:1-16.

Nordestgaard BG. The vascular endothelial barrier – selective retention of lipoproteins. *Curr Opin Lipidol* 1996;7:269-273.

O´Meara NM, Lewis GF, Cabana VG, Iverius PH, Getz GS, Polonsky KS. Role of basal triglyceride and high density lipoprotein in determination of postprandial lipid and lipoprotein responses. *J Clin Endocrinol Metab* 1992;75:465-471.

Orchard TJ, Eichner J, Kuller LH, Becker DJ, McCallum LM, Grandits GA. Insulin as a predictor of coronary heart disease: interaction with apolipoprotein E phenotype. A report from the Multiple Risk Factor Intervention Trial. *Ann Epidemiol* 1994;4:40-45.

Orchard TJ, Virella G, Forrest KY, Evans RW, Becker DJ, Lopes-Virella MF. Antibodies to oxidized LDL predict coronary artery disease in type I diabetes: a nested case control study from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 1999;48:1454-1458.

Ordovas JM, Lopez-Miranda J, Mata P, Perez-Jimenez, Lichtenstein AH, Schaefer EJ. Gene-diet interaction in determining plasma lipid response to dietary intervention. *Atherosclerosis* 1995;118:S11-17.

Ordovas JM, Mooser V. The APOE locus and the pharmacogenetics of lipid response. *Curr Opin Lipidol* 2002;13:113-117.

Otvos JD, Jeyarajah EJ, Bennett DW, Krauss M. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin Chem* 1992;38:1632-1638.

Packard C, Caslake M, Shepherd J. The role of small, dense low density lipoprotein (LDL): a new look. *Int J Cardiol* 2000;74:S17-22.

Pajunen P, Nieminen MS, Taskinen MR, Syvänne M. Quantitative comparison of angiographic characteristics of coronary artery disease in patients with noninsulin-dependent diabetes mellitus compared with matched nondiabetic control subjects. *Am J Cardiol* 1997;80:550-556.

Pajunen Pia. Studies on the severity and extent of coronary atherosclerosis utilizing computer-assisted quantification of coronary angiograms. With special reference to diabetes mellitus. Academic dissertation. University of Helsinki, Faculty of Medicine, Department of medicine, Division of Cardiology, April 2002.

Paragh G, Balogh Z, Seres I, Harangi M, Boda J, Kovacs P. Effect of gemfibrozil on HDL-associated serum paraoxonase activity and lipoprotein profile in patients with hyperlipidemia. *Clin Drug Invest* 2000;19:277-282.

Parra HJ, Mezdour H, Ghalim N, Bard JM, Fruchart JC. Differential electroimmunoassay of human LpA-I lipoprotein particles on ready-to-use plates. *Clin Chem* 1990;36:1431-1435.

Patsch JR, Miesenböck G, Hopferwieser T, Muhlberger V, Knapp E, Dunn JK, Gotto AM Jr, Patsch W. Relation of triglyceride metabolism and coronary artery disease: studies in the postprandial state. *Arterioscler Thromb* 1992;12:1336-1345.

Pedersen TR, Olsson AG, Faergeman O, Kjekshus J, Wedel H, Berg K, Wilhelmsen L, Haghfelt T, Thorgeirsson G, Pyörälä K, Miettinen T, Christophersen B, Tobert JA, Musliner TA, Cook TJ. Lipoprotein changes and reduction in the incidence of major coronary heart disease events in the Scandinavian Simvastatin Survival Study (4S). *Circulation* 1998;97:1453-1460.

Pedro-Botet J, Schaefer EJ, Bakker-Arkema RG, Black DM, Stein EM, Corella D, Ordovas JM. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. *Atherosclerosis* 2001;158:183-193.

Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986;74:1399-1406.

Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004;291:1730-1737.

Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics* 1996;33:498-509.

Prior JO, Quinones MJ, Hernandez-Pampaloni M, Facta AD, Schindler TH, Sayre JW, Hsueh WA, Schelbert HR. Coronary circulatory dysfunction in insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. *Circulation* 2005;111:2291-2298.

Pundziute G, Schuijf JD, Jukema JW, de Roos A, van der Wall E, Bax JJ. Advances in the noninvasive evaluation of coronary artery disease with multislice computed tomography. *Expert Rev Med Devices* 2006;3:441-451.

Pyörälä K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev* 1987;3:463-524.

Pyörälä M, Miettinen H, Laakso M, Pyörälä K. Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men. The 22-year follow-up results of the Helsinki Policemen Study. *Circulation* 1998;98:398-404.

Rader DJ, Hoeg JM, Brewer HB Jr. Quantification of plasma lipoproteins in the primary and secondary prevention of coronary artery disease. *Ann Intern Med* 1994;20:1012-1025.

Raffai RL, Loeb SM, Weisgraber KH. Apolipoprotein E promotes the regression of atherosclerosis independently of lowering plasma cholesterol levels. *Arterioscler Thromb Vasc Biol* 2005;25:436-441.

Reaven GM. Banting Lecture 188. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595-1607.

Redgrave TG, Carlson LA. Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man. *J Lipid Res* 1979;20:217-229.

Reiber JHC, van der Zwet PMJ, von Land CD, Koning G, van Meurs B, Buis B, van Voorthuisen AE. Quantitative coronary arteriography: equipment and technical requirements. In: Reiber JCH, Serreuys PW, eds. Advances in quantitative coronary arteriography. Dordecht: Kluwer Academic Publishers, 1993:75-111.

Resnick HE, Jones K, Ruotolo G, Jain AK, Henderson J, Lu W, Howard BV. Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic American Indians. The Strong Heart Study. *Diabetes Care* 2003;26:861-867.

Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping. *Pharmacogenetics* 1999;9:745-753.

Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. *Q J Med* 2006;99:1-14.

Roman MJ, Pickering TG, Pini R, Schwartz JE, Devereux RB. Prevalence and determinants of cardiac and vascular hypertrophy in hypertension. *Hypertension* 1995;26:369-373.

Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999;340:115-126.

Ruan H, Lodish HF. Regulation of insulin sensitivity by adipose tissue-derived hormones and inflammatory cytokines. *Curr Opin Lipidol* 2004;15:297-302.

Rye KA, Clay MA, Barter PJ. Remodelling of high density lipoproteins by plasma factors. *Atherosclerosis* 1999;145:227-238.

Sacks FM, Campos H. Low-density lipoprotein size and cardiovascular disease: a reappraisal. *J Clin Endocr Metab* 2003;88:4525-4532.

Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssönen K, Palinski W, Witztum JL. Autoantibody against oxidized LDL and progression of carotid atherosclerosis. *Lancet* 1992;339:883-887.

Salonen R, Salonen JT. Determinants of carotid intima-media thickness: a population-based ultrasonography study in eastern Finnish men. *J Intern Med* 1991;229:225-231.

Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, Boekholdt M, Khaw KT, Gudnason W. Triglycerides and the risk of coronary heart disease. 10158 incident cases among 262 525 participants in 29 western prospective studies. *Circulation* 2007;115:450-458.

Sasso FC, Carbonara O, Nasti R, Campana B, Marfella R, Torella M, Nappi G, Cozzolino D. Glucose metabolism and coronary heart disease in patients with normal glucose tolerance. *JAMA* 2004;291:1857-1863.

Satoh H, Terada H, Uehara A, Katoh H, Matsunaga M, Yamazaki K, Matoh F, Hayashi H. Post-challenge hyperinsulinemia rather than hyperglycemia is associated with the severity of coronary artery disease in patients without a previous diagnosis of diabetes mellitus. *Heart* 2005;91:731-736.

Sawamura T, Kume N, Aoyma T, Moriwaki H, Hishikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T, Masaki T. An endothelial receptor for oxidized low-density lipoprotein. *Nature* 1997;386:73-77.

Sawayama Y, Shimizu C, Maeda N, Tatsukawa M, Kinukawa N, Koyanagi S, Kashiwagi S, Hayashi J. Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. *J Am Coll Cardiol* 2002;39:610-616.

Schaefer EJ, Levy RI, Anderson DW, Danner RN, Rewer HB Jr, Blackwelder WC. Plasma triglycerides in regulation of HDL cholesterol levels. *Lancet* 1978;2:391-393.

Schildkraut JM, Myers RH, Cupples LA, Kiely DK, Kannel WB. Coronary risk associated with age and sex of parental heart disease in the Framingham Study. *Am J Cardiol* 1989;64:555-559.

Schmidt H, Schmidt R, Niederkorn K, Gradert A, Schumacher M, Watzinger N, Hartung HP, Kostner GM. Paraoxonase PON1 polymorphism Leu-Met54 is associated with carotid atherosclerosis: results of the Austrian Stroke Prevention Study. *Stroke* 1998;29:2043-2048.

Schoenhagen P, Ziada KM, Vince DG, Nissen SE, Tuzcu M. Arterial remodeling and coronary artery disease: the concept of dilated versus obstructive coronary atherosclerosis. *J Am Coll Cardiol* 2001;38:297-306.

Schreiner PJ, Heiss G, Tyroler HA, Morrisett JD, Davis CE, Smith R. Race and gender differences in the association of Lp(a) with carotid artery wall thickness. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol* 1996;16:471-478.

Schwartz JN, Kong Y, Hackel DB, Bartel AG. Comparison of angiographic and postmortem findings in patients with coronary artery disease. *Am J Cardiol* 1975;36:174-178.

Selzer A. On the limitation of therapeutic intervention trials in ischemic heart disease: a clinician´s viewpoint. *Am J Cardiol* 1982;49:252-255.

Serhatlioglu S, Gursu MF, Gulcu F, Canatan H, Godekmerdan. Levels of paraoxonase and arylesterase activities and malondialdehyde in workers exposed to ionizing radiation. *Cell Biochem Funct* 2003;21:371-375.

Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 1998;394:284-287.

Shin HK, Kim YK, Kim KY, Lee JH, Hong KW. Remnant lipoprotein particles induce apoptosis in endothelial cells by NAD(P)H oxidase-mediated production of superoxide and cytokines via lecitin-like oxidized low-density lipoprotein receptor-1 activation: prevention by cilostazol. *Circulation* 2004;109:1022-1028.

Shinozaki K, Hattori Y, Suzuki M, Hara Y, Kanazawa A, Takaki H, Tsushima M, Harano Y. Insulin resistance as an independent risk factor for carotid artery wall intima media thickening in vasospastic angina. *Arterioscler Thromb Vasc Biol* 1997;17:3302-3310.

Siggins S, Jauhiainen M, Olkkonen VM, Tenhunen J, Ehnholm C. PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma. *J Lipid Res* 2003;44:1698-1704.

Simes RJ, Marschner IC, Hunt D, Colquhoun D, Sullivan D, Stewart RA, Hague W, Keech A, Thompson P, White H, Shaw J, Tonkin A. Relationship between lipid levels and clinical outcomes in the Long-term Intervention with Pravastatin in Ischemic Disesase (LIPID) Trial: to what extent is the reduction in coronary events with pravastatin explained by on-study lipid levels? *Circulation* 2002;105:1162-1169.

Simon A, Gariepy J, Chironi G, Megnien JL, Levenson J. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. *J Hypertens* 2002;20:159-169.

Simons LA, Dwyer T, Simons J, Bernstein L, Mock P, Poonia NS, Balasubramaniam S, Baron D, Branson J, Morgan J, Roy P. Chylomicrons and chylomicron remnants in coronary artery disease: a case-control study. *Atherosclerosis* 1987;65:181-189.

Simpson HS, Williamson CM, Olivecrona T, Pringle S, Maclean J, Lorimer AR, Bonnefous F, Bogaievsky Y, Packard CJ, Shepherd J. Postprandial lipemia, fenofibrate and coronary artery disease. *Atherosclerosis* 1990;85:193-202.

Simpson NE. Serum arylesterase levels of activity in twins and their parents. *Am J Hum Genet* 1971;23:375-382.

Skoglund-Andersson C, Tang R, Bond MG, de Faire U, Hamsten A, Karpe F. LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. *Arterioscler Thromb Vasc Biol* 1999;19:2422-2430.

Slooter AJ, Bots ML, Havekes LM, del Sol AI, Cruts M, Grobbee DE, Hofman A, Van Broeckhoven C, Witteman JC, van Duijn CM. Apolipoprotein E polymorphism and carotid artery atherosclerosis. The Rotterdam Study. *Stroke* 2001;32:1947-1952.

Smit M, de Knijff P, Rosseneou M, Bury J, Klasen E, Frants E, Havekes L. Apolipoprotein E polymorphism in the Netherland and its effect on plasma lipid and apolipoprotein levels. *Hum Genet* 1988;80:287-292.

Smith GD, Shipley MJ, Marmot MG, Rose G. Plasma cholesterol concentration and mortality. The Whitehall Study. *JAMA* 1992;267:70-76.

Smith SC, Greenland P, Grundy SM. Prevention conference V. Beyond secondary prevention: identifying the high-risk patient for primary prevention. Executive summary. *Circulation* 2000;101:111-116.

Smith SC. Current and future directions of cardiovascular risk prediction. *Am J Cardiol* 2006;97:28-32.

Smith SJ, Cooper GR, Myers GL. Sampson EJ. Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. *Clin Chem* 1993;3:1012-1022.

Sniderman AD, Cianflone K. Measurement of apolipoproteins: time to improve the diagnosis and treatment of the atherogenic dyslipoproteinemias. *Clin Chem* 1996;42:489-491.

Solberg LA, Eggen DA. Localization and sequence of development of atherosclerotic lesions in the carotid and vertebral arteries. *Circulation* 1971;43:711-724.

Sones FM. Acquired heart disease: symposium on present and future of cineangiography. *Am J Cardiol* 1959;3:710.

Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk of coronary heart disease. *Ann Int Med* 2004;141:137-147.

Sozmen EY, Mackness B, Sozmen B, Durrington P, Girgin FK, Aslan L, Mackness M. Effect of organophosphate intoxication on human serum paraoxonase. *Hum Exp Toxicol* 2002;21:247-252.

Stampfer MJ, Sacks FM, Salvini S, Willett WC, Henneckens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med* 1991;325:373-381.

Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: a report from the committee on vascular lesions of the council on arteriosclerosis, American Heart Association. Special report. *Circulation* 1994;89:2462-2478.

Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the committee on vascular lesions of the council on arteriosclerosis, American Heart Association. Special report. *Circulation* 1995;92:1355-1374.

Stehbens WE. General features, structure, topography and adaption of the circulatory system. Vascular pathology. Ed. Stehbens WE, Lie JT. London, UK: Chapman & Hall Medical, 1995:1-20.

Stein O, Stein Y. Atheroprotective mechanisms of HDL. *Atherosclerosis* 1999;144:285-301.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915-924.

Steinberg D. Oxidative modification of LDL and atherogenesis. *Circulation* 1997;95:1062-1071.

Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron A. Obesity/insulin resistance is associated with endothelial function. *J Clin Invest* 1996;97:2601-2610.

Stengard JH, Weiss KM, Sing CF. An ecological study of association between coronary heart disease mortality rates in men and the relative frequencies of common allelic variations in the gene coding for apolipoprotein. *E Hum Genet* 1998;103:234-241.

Stern MP. Do non-insulin dependent diabetes mellitus and cardiovascular disease share common antecedents? *Ann Intern Med* 1996;124:110-116.

Stiel GM, Stiel LS, Schofer J, Donath K, Mathey DG. Impact of compensatory enlargement of atherosclerotic coronary arteries on angiographic assessment of coronary heart disease. *Circulation* 1989;80:1603-1609.

Sullivan DR, Marwick TH, Freedman SB. A new method of scoring coronary angiograms to reflect extent of coronary atherosclerosis and improve correlation with major risk factors. *Am J Heart* 1990;119:1262-1267.

Sundell J, Knuuti J. Insulin and myocardial blood flow. *Cardiovasc Res* 2003;57:312-319.

Sutherland WH, Walker RJ, de Jong SA, van Rij AM, Phillips V, Walker HL. Reduced postprandial serum paraoxonase activity after a meal rich in used cooking fat. *Arterioscler Thromb Vasc Biol* 1999;19:1340-1347.

Suzuki M, Shinozaki K, Kanazawa A, Hara Y, Hattori Y, Tsushima M, Harano Y. Insulin resistance as an independent risk factor for carotid wall thickening. *Hypertension* 1996;28:593-598.

Syvänne M, Vuorinen-Markkola H, Hildén H, Taskinen MR. Gemfibrozil reduces postprandial lipemia in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb* 1993;13:286-295.

Syvänne M, Hilden H, Taskinen MR. Abnormal metabolism of postprandial lipoproteins in patients with non-insulin-dependent diabetes mellitus is not related to coronary artery disease. *J Lipid Res* 1994;35:15-26(a).

Syvänne M, Nieminen MS, Frick MH. Accuracy and precision of quantitative arteriography in the evaluation of coronary artery disease after coronary bypass surgery. *Int J Cardiac Imaging* 1994;10:243-252(b).

Takeichi S, Yukawa N, Nakajima Y, Osawa M, Saito T, Seto Y, Nakano T, Saniabadi AR, Adachi M, Wang T, Nakajima K. Association of plasma triglyceride-rich lipoprotein remnants with coronary atherosclerosis in cases of sudden death. *Atherosclerosis* 1999;142:309-315.

Tall A. Plasma lipid transfer proteins. *Annu Rev Biochem* 1995;64:235-257.

Talmud PJ, Hawe E, Miller GJ, Humphries SE. Non-fasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease risk in middle-aged UK men. *Arterioscler Throm Vasc Biol* 2002;22:1918-1923.

Tanaka H, Nishino M, Ishida M, Fukunaga R, Sueyoshi K. Progression of carotid atherosclerosis in Japanese patients with coronary artery disease. *Stroke* 1992;23:946-951.

Tardif JC, Gregoire J, Schwartz L, Title L, Laramee L, Reeves F, Lesperance J, Bourassa MG, LÁllier PL, Glass M, Lambert J, Guertin MC; for the Canadian Antioxidant Restenosis Trial (CART-1) Investigators. Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation* 2003;107:552-558.

Taskinen MR, Kuusi T, Helve E, Nikkilä EA, Yki-Järvinen H. Insulin therapy induces antiatherogenic changes of serum lipoproteins in noninsulin-dependent diabetes. *Arteriosclerosis* 1988;8:168-177.

Teo KK, Ounpuu S, Hawken S, Pandey MR, Valentin V, Hunt D, Diaz R, Rashed W, Freeman R, Jiang K, Zhang X, Yusuf S; INTERHEART Study investigators. Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study. *Lancet* 2006;19:647-658.

Terry JG, Howard G, Mercuri M, Bond MG, Crouse JR 3rd. Apolipoprotein E polymorphism is associated with segment-specific extracranial carotid artery intima-media thickening. *Stroke* 1996;27:1755-1759.

Thomas CS, Cherian G, Hayat N, Varma LK. Angiographic comparison of coronary artery disease in Arab women with and without type II diabetes mellitus. *Med Princ Pract* 2002;11:63-68.

Tikkanen MJ, Huttunen JK, Ehnholm C, Pietinen P. Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. *Arteriosclerosis* 1990;10:285-288.

Tomas M, Senti M, Garcia-Faria F, Vila J, Torrents A, Covas M, Marrugat J. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolaemic patients. *Arterioscler Thromb Vasc Biol* 2000;20:2113-2119.

Topol EJ, Nissen SE. Our preoccupation with coronary luminology: the dissociation between clinical and angiographic findings in ischemic heart disease. *Circulation* 1995;92:2333-2342.

Toshima S, Hasegawa A, Kurabayashi M, Itabe H, Takano T, Sugano J, Shimamura K, Kimura J, Michishita I, Suzuki T, Nagai R. Circulating oxidized low density lipoprotein levels: a biochemical risk marker for coronary heart disease. *Arterioscler Thromb Vasc Biol* 2000;20:2243-2247.

Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M, Hernandez R, Jaff M, Kownator S, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E, Woo KS, Zannad F, Zureik M. Mannheim carotid intima-media thickness consensus (2004-2006). *Cerebrovasc Dis* 2004;18:346-349.

Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ. Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am J Med* 2001;110:103-110.

Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. *Circulation* 2001;103:1930-1932.

Tsuchihashi K, Hikita N, Hase M, Agata J, Saitoh S, Nakata T, Ura N, Shimamoto K. Role of hyperinsulinemia in atherosclerotic coronary arterial disease: studies of semi-quantitative coronary angiography. *Intern Med* 1999;38:691-697.

Turay J, Grniakova V, Valka J. Changes in paraoxonase and apolipoprotein A-I, B, C-III and E in subjects with combined familial hyperlipoproteinaemia treated with ciprofibrate. *Drugs Exp Clin Res* 2000;26:83-88.

Turban S, Fuentos F, Ferlic L, Brugada R, Gotto AM, Ballantyne LF, Marian AJ. A prospective study of paraoxonase gene Q/R192 polymorphism and severity, progression and regression of coronary atherosclerosis, plasma lipid levels, clinical events and response to fluvastatin. *Atherosclerosis* 2001;154:633-640.

Tward A, Xia YR, Wang XP, Shi YS, Park C, Castellani LW, Lusis AJ, Shih DM. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation* 2002;106:484-490.

Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration. A characteristic feature of the atherogenic lipoprotein phenotype. *Circulation* 2004;109:1918-1925.

Uusitupa MI, Niskanen L, Luoma J, Vilja P, Mercuri M, Rauramaa R, Ylä-Herttuala S. Autoantibodies against oxidized LDL do not predict atherosclerotic vascular disease in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1996;16:1236-1242.

Vakkilainen J, Jauhiainen M, Ylitalo K, Nuotio IO, Viikari JSA, Ehnholm C, Taskinen MR. LDL particle size in familial combined hyperlipidemia: effects of serum lipids, lipoprotein-modifying enzymes, and lipid transfer proteins. *J Lipid Res* 2002;43:598-603(a).

Vakkilainen J, Mero N, Schweizer A, Foley JE, Taskinen MR. Effects of nateglinide and glibenclamide on postprandial lipid and glucose metabolism in type 2 diabetes. *Diabetes Metab Res Rev* 2002;18:484-490(b).

Valabhji J, McColl AJ, Schachter M, Dhanjil S, Richmonds W, Elkeles RS. High-density lipoprotein composition and paraoxonase activity in type I diabetes. *Clinical Science* 2001;101:659-670.

van der Gaag MS, van Tol A, Scheek LM, James RW, Urgert R, Schaafsma G, Hendricks HF. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis* 1999;147:405-410.

van de Vijver LP, Steyger R, van Poppel G, Boer JM, Kruijssen DA, Seidell JC, Princen HM. Autoantibodies against MDA-LDL in subjects with severe and minor atherosclerosis and healhty population controls. *Atherosclerosis* 1996;122:245-253.

Vavuranakis M, Stefanadis C, Toutouzas K, Pitsavos C, Spanos V, Toutouzas P. Impaired compensatory artery enlargement in atherosclerosis contributes to the development of coronary artery stenosis in diabetic patients. An in vivo intravascular ultrasound study. *Eur Heart J* 1997;18:1090-1094.

Vega GL, Grundy SM. Does measurement of apolipoprotein B have a place in cholesterol management? *Arteriosclerosis* 1990;10:668-671.

von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol* 2001;21:13-27.

Wallace TW, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-1495.

Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet* 2001;358:2026-2033.

Wallenfeldt K, Fagerberg B, Wikstrand J, Hulthe J. Oxidized low-density lipoprotein in plasma is a prognostic marker of subclinical atherosclerosis development in clinically healhty men. *J Intern Med* 2004;256:413-420.

Waller BF, Palumbo PJ, Liet J, Roberts WC. Status of the coronary arteries at necropsy in diabetes mellitus with onset after age 30 years. Analysis of 229 diabetic patients with and without clinical evidence of coronary heart disease and comparison to 183 control subjects. *Am J Med* 1980;69:498-506.

Wang CS, McConathy WJ, Kloer HU, Alaupovic P. Modulation of lipoprotein lipase activity by apolipoproteins. *J Clin Invest* 1985;75:384-390.

Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, Navab M. Protective effect of high-density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low-density lipoprotein. *J Clin Invest* 1995;96:2882-2891.

Weintraub MS, Grosskopf I, Rassin T, Miller H, Charach G, Rotmensch HH, Liron M, Rubinstein A, Iaina A. Clearance of chylomicron remnants in normolipidemic patients with coronary artery disease: case control study over three years. *Br Med J* 1996;312:935-939.

Welin L, Eriksson H, Larsson B, Ohlson LO, Svardsudd K, Tibblin G. Hyperinsulinemia is not a major coronary risk factor in elderly men. The study of men born in 1913. *Diabetologia* 1992;35:766-770.

Wendelhag I, Gustavsson T, Suurkula M, Berglund G, Wikstrand J. Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. *Clin Physiol* 1991;11:567-577.

Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J. Four paraoxonase gene polymorphisms in 11 212 cases of coronary heart disease and 12 786 controls: meta-analysis of 43 studies. *Lancet* 2004;363:689-695.

Williams CM. Cardiovascular risk factors in women. *Proc Nutr Soc* 1997;56:383-391.

Wilson DE, Chan IF, Buchi KN, Horton SC. Postchallenge plasma lipoprotein retinoids:chylomicron remnants in endogenous hypertriglyceridemia. *Metabolism* 1985;34:551-558.

Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality; the Framingham Heart Study. *Arteriosclerosis* 1988;8:737-741.

Wilson PW, Myers RH, Larson MG, Ordovas JM, Wolf PA, Schaefer EJ. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease. The Framingham Offspring Study. *JAMA* 1994;272:1666-1671.

Witztum JL, Steinberg D. The oxidative modification hypothesis of atherosclerosis. Does it hold for humans? *Trends Cardiovasc Med* 2001;11:93-102.

Wofford JL, Kahl FR, Howard GR, McKinney WM, Toole JF, Crouse JR 3rd. Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. *Arterioscler Thromb* 1991;11:1786-1794.

Wolf PA, D´Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. *Stroke* 1991;22:312-318.

Wolf PA, D´Agostino RB, O´Neal MA, Sytkowski P, Kase CS, Belanger AJ, Kannel WB. Secular trends in stroke incidence and mortality: the Framingham Study. *Stroke* 1992;23:1551-1555.

Wong M, Edelstein J, Wollman J, Bond MG. Ultrasonic-pathological comparison of the human arterial wall. Verification of intima-media thickness. *Arterioscler Thromb* 1993;13:482-486.

World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. WHO/NCD/NCS/99.2. Geneva: World Health Organization; 1999.

Writing Group for the Women´s Health Initiative (WHI) investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women´s Health Initiative Randomized Controlled Trial. *JAMA* 2002;288:321-333.

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Guto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411-415.

Yanase M, Takatsu F, Tagawa T, Kato T, Arai K, Koyasu M, Horibe H, Nomoto S, Takemoto K, Shimizu S, Watarai M. Insulin resistance and fasting hyperinsulinemia are risk factors for new cardiovascular events in patients with prior coronary artery disease and normal glucose tolerance. *Circ J* 2004;68:47-52.

Zanzonico P, Rothenberg LN, Strauss HW. Radiation exposure of computed tomography and direct intracoronary angiography: risk has its reward. *J Am Coll Cardiol* 2006;47:1846-1849.

Ylitalo K, Syvänne M, Salonen R, Nuotio I, Taskinen M-R, Salonen JT. Carotid artery intima-media thickness in Finnish families with familial combined hyperlipidemia. *Atherosclerosis* 2002;162:171-178.

Ylitalo Kati. Pathophysiology of adipose tissue metabolism and atherosclerosis in familial combined hyperlipidemia. Academic dissertation. University of Helsinki, Faculty of Medicine, Department of Medicine, Division of Cardiology, December 2001.

Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 1989;84:1086-1095.

Yuhanna IS, Zhu Y, Cox BE, Hahner LD, Osborne-Lawrence S, Lu P, Marcel YL, Anderson RG, Mendelsohn ME, Hobbs HH, Shaul PW. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med* 2001;7:853-857.

Zilversmit. Atherogenesis: a postprandial phenomenon. *Circulation* 1979;60:473-485.

Zir LM, Miller SW, Dinsmore RE, Gilbert JP, Harthorne JW. Interobserver variability in coronary angiography. *Circulation* 1976;53:627-632.